THE AMERICAN JOURNAL OF Clinical Nutrition

JULY–AUGUST VOLUME 9, NUMBER 4

Symposium on Neurological and Hepatic Complications of Alcoholism

On the Etiology of the Alcoholic Neurologic Diseases

With Special Reference to the Role of Nutrition

MAURICE VICTOR, M.D.* AND RAYMOND D. ADAMS, M.D.†

THE main purpose of this report is to summarize a series of investigations which have been made of the causes of the neurologic diseases associated with alcoholism. Although the studies are not complete, the principal findings are fairly definite and of general interest. Doubtless they would be more convincing if extended over a longer period of time in order to include a larger number of carefully observed patients. However, the paucity of certain types of clinical material makes this impractical, and we consider this an appropriate time to terminate this part of our clinical investigation and to report our findings.

In the presentation of our data we believe it important to describe briefly the clinical conditions which have been investigated. These conditions fall roughly into the four categories listed herein. This division of the clinical material is made only for the purpose of facilitating their exposition. A definitive classification, based on the results of these studies, will be offered at the conclusion of the article.

Our observations have been made on the following types of clinical case: (1) alcoholic intoxication—the drunken, combative, comatose states; (2) the tremulous, hallucinatory, convulsive delirious states; (3) diseases associated with protracted alcoholism and nutritional depletion and characterized by specifically localized, bilaterally symmetrical neuropathologic changes: the Wernicke-Korsakoff syndrome, polyneuropathy, alcoholic or nutritional amblyopia, pellagra with neurologic disorder, alcoholic cerebellar degeneration,

From the Department of Neurology and Psychiatry (Neuropathology), Harvard Medical School and the Neurology Service, Massachusetts General Hospital, Boston, Massachusetts.

* Assistant Clinical Professor of Neurology; †Bullard Professor of Neuropathology, Harvard Medical School, Boston, Massachusetts.

This study was supported in part by grant B-1906 from the National Institute of Neurological Diseases and Blindness.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

Marchiafava-Bignami disease, and central pontine myelinolysis; (4) diseases related to alcoholic cirrhosis.

Alcoholic Intoxication

Alcoholic intoxication, inebriation or drunkenness is by far the most common clinical manifestation of alcoholism. With the exception of coma, or of certain unusual and extreme alterations of behavior, such as combativeness, a marked paranoid tendency or other antisocial activities (so-called pathologic intoxication), this state presents little problem to the physician either in diagnosis or management. The various manifestations of alcoholic intoxication appear to result from the depressant action of the drug on neurones in certain selected parts of the nervous system, possibly the upper brain stem and diencephalon, acting in a manner akin to barbiturate and inhalation anesthesia. Unlike these agents, however, the margin between deep surgical anesthesia and depression of respiration is very narrow, and now and again alcoholic narcosis may prove fatal. The part played by pre-existent psychiatric disease in the deviate forms of pathologic alcoholic intoxication is by no means clear, despite the assumption of some physicians that the basic personality structure determines the form of alcoholic intoxication. Pathologic intoxication has also been ascribed to constitutional differences in the susceptibility to alcohol, to previous craniocerebral trauma and to an underlying epileptic predisposition, but there is little factual data to support any of these beliefs.

The relationship of alcohol to nervous disorder is so obvious in this group of patients, and definable nutritional deficiency, at least of the type that could be investigated by chemical technics in our laboratory, is so improbable, that we have undertaken no special studies of this category of alcoholic disease.

The Tremulous, Hallucinatory, Convulsive, Delirious States

These are a closely related group of symptoms which may occur in relatively pure form, but more frequently, in combination with one another. The prototype of the patients afflicted with these symptoms is the so-called spree or periodic drinker, although the steady drinker is not immune, if, for some reason, alcohol has been withdrawn.

A state of *tremulousness*, often combined with general irritability, nausea or vomiting, is the single most frequent casue for admission of an alcoholic patient to the hospital. This syndrome is commonly referred to as "the shakes." The symptoms first appear after several days or weeks of drinking and are invariably worse in the morning, following the short period of abstinence occasioned by a night's sleep. A drink or two will suppress the tremor and nausea, but they return the next morning with increasing persistence and severity. Upon termination of the spree, the symptoms become greatly augmented, reaching their peak of intensity within twenty-four hours after the complete cessation of drinking. At this stage the patient presents a familiar clinical picture, characterized by varying degrees of weakness, tremor, anorexia and insomnia and, in about 10 per cent of cases, by one or more seizures—the so-called *rum fits*. Symptoms of disordered sense perception are very common, affecting about a quarter of the tremulous patients, and take the form of misinterpretations, misidentifications and *hallucinations*.

Delirium tremens, characterized by intense psychomotor and speech activity, autonomic overactivity, in the form of sweating, tachycardia, dilated pupils and fever, gross disorders of sense perception and profound confusion, appears to be a more severe form of the same disease state. Defined in this way, delirium tremens is a serious illness, with a mortality rate of 15 per cent, in our experience.[1] Death may occur not only in the cases complicated by trauma and infection, but also in the absence of these factors, the patient dying suddenly in a state of peripheral circulatory collapse or of hyperthermia.

Auditory hallucinosis in the alcoholic patient is an illness closely related although distinguishable from delirium tremens. It consists of pure auditory hallucinations without confusion or clouding of the sensorium. The

hallucinations are intensely vivid and usually take the form of accusative, threatening voices arising from some place in the immediate environment; the patients react with extreme fright or some other emotion that is altogether appropriate to the hallucinatory content. In the acute stages of this illness suicide may actually be committed to avoid the consequence of the imaginary accusations. The syndrome usually clears within a few days, with the restoration of normal mentaton, but one of every eight or nine of our patients have drifted into a chronic hallucinatory state, which eventually becomes indistinguishable from paranoid schizophrenia, even though schizophrenic traits had not existed before the onset of the illness or in its early stages.[2] Patients with the chronic form of the illness constitute a significant portion of the mental hospital population.

One's first reaction is to regard these several illnesses as manifestations of the toxic effects of alcohol. A moment's reflection, however, leads one to doubt this simple explanation. The symptoms of toxicity, consisting of slurred speech, truculence, staggering, stupor, coma and so forth are in themselves quite distinctive and differ from the symptom complex of tremor, hallucinosis, fits and delirium. Further, it may be shown that the former symptoms are associated with a high level of alcohol in the blood in contrast to a low or negligible level in the second group. Whereas the continued use of alcohol simply intensifies the symptoms of intoxication, it actually tends to nullify symptoms such as nausea, vomiting, tremor and hallucinations, and even full-blown delirium tremens may subside in an ordinary manner while the patient is receiving a pint or more of whiskey daily.

In the medical literature one finds several other theories concerning the mechanism of delirium tremens and related disorders. A specific adrenal insufficiency has been postulated[3] although in our own experience, the administration of adrenal cortical extract and ACTH have not altered the course of recovery in these patients in any way that could not be accounted for by the natural history of the disease.[1] Other constitutional, traumatic or infectious factors have been invoked but can hardly be considered more than predisposing causes. Similarly, hypoglycemia, a deficiency of blood sodium, potassium, chloride and magnesium appear to be variable and inconstant abnormalities in this group of patients.

The role of nutrition in the genesis of delirium tremens and related disorders has been under scrutiny for many years by many workers. In evaluating this factor we have secured several types of data: (1) dietary history and nutritional status on examination; (2) observations on the natural history of the illness; and (3) observations on the mode of recovery under strict dietary control.

(1) A series of 488 patients with delirium tremens and related disorders, personally examined, were reviewed in order to determine the patient's dietary intake in the weeks preceding hospital entry and their nutritional status when first seen. Only 213 of these were considered acceptable for analysis, since only in this number had the examination been complete and the dietary history trustworthy, i.e., substantiated by relatives and friends who were judged to be reliable.

Of the 213 patients, the dietary intake was grossly inadequate in 148 patients. Practically all of these 148 patients were spree drinkers; characteristically they ate in diminishing quantities for the first few days of the spree, then little or nothing for days on end, or even for a week or two. On examination, thirty-seven of the 148 exhibited signs which could possibly be attributed to malnutrition. Fourteen showed a marked generalized thinness, due to a loss of both subcutaneous fat and of muscle bulk. Dryness, coarseness and loss of turgor of the skin, as well as other lesions such as acne rosacea and rhinophyma were observed in ten patients. In a similar number there was evidence of advanced liver disease, i.e., jaundice, ascites and "spider" angiomas. In thirty-four patients, initial examination disclosed enlargement of the liver, but this was reversible in all cases and probably represented a "fatty hepatosis" and not cirrhosis. The signs of polyneuropathy were present in four patients, but were not

TABLE I

The Mode of Recovery of Thirty Patients with Delirium Tremens Who Were Denied Food or Vitamins During the Course of Their Illness

No. of Patients	Therapeutic Regimen	Duration of Delirium Tremens
10	Water *ad libitum*	24–96 hours
10	5% glucose/saline or 5% glucose/water intravenously	24–96 hours
9	90 proof whiskey* (45 ml. every 4 hours)	72–96 hours
1	Kempner rice diet†	48 hours

* Seven patients given whiskey recovered within seventy-two hours; two others showed no improvement after seventy-two hours, and in them the whiskey was discontinued and replaced with paraldehyde; both recovered after another twenty-four hours.

† This diet is the one used by Dr. Walter Kempner of Duke University. It consists of rice, fruit, fruit juices, sugar and honey, and contains the following nutrients:

Calories	2,001	Iron	9 mg.
Protein	12 gm.	Vitamin	2,200 I.U.
Fat	1 gm.	Ascorbic acid	115 gm.
Carbohydrate	486 gm.	Thiamine	0.4 mg.
Calcium	0.2 gm.	Riboflavin	0.3 mg.
Phosphorus	0.4 gm.	Niacin	5 mg.

severe, consisting only of a loss of knee and ankle jerks and a blunting of sensation in the feet and legs.

On the other hand, the histories disclosed that in twenty of the 213 patients, the dietary intake was probably adequate, and in the remaining forty-five it was decidedly so, i.e., they took three meals of a balanced ration each day. Furthermore, these forty-five patients appeared well nourished, and the only questionable feature of the examination was the occurrence in three patients of hepatomegaly, which was readily reversible in each instance.

(2) A number of observations made in the course of the routine ward management of patients with delirium tremens and related disorders threw further light on the role of nutritional factors in the causation of this group of diseases. Delirium tremens developed in ten patients after they had been hospitalized for periods of one to five days, during which time they had received a nutritious diet and supplementary vitamins. Similarly in three patients with acute auditory hallucinations the chronic form of the illness developed over a three to four week period, despite the administration of a good diet and supplementary vitamins during this time. Conversely, in six patients, a state of tremor and hallucinosis subsided in an ordinary manner, i.e., in two to four days, despite the fact that they had been anorectic and had taken no food or vitamins throughout the period of their recovery.

TABLE II

Mode of Recovery of Ten Patients with Acute Auditory Hallucinosis Who Were Denied Food or Vitamins During the Course of Their Illness

No. of Patients	Therapeutic Regimen	Duration of Hallucinations
3	Water *ad libitum*	48 hours 3 days 7 days
6	Dextrose (20%) and water	24 hours 24 hours 48 hours 48 hours 3 days 8 days
1	Dextrose, water and whiskey (45 cc. every 3 hours x 10)	3½ days

(3) Direct observation of the neurologic syndromes were made under conditions of strict dietary control. In thirty patients with delirium tremens, as part of this planned clinical investigation, all food and vitamins were withheld until the termination of the illness. Paraldehyde, 10 ml. orally, was given when necessary except to those patients who received whiskey as a special part of the clinical experiment to be described below. The results of this experiment are summarized in Table I.

It will be seen that all the experimental patients recovered in twenty-four to ninety-six hours. Such a rate of recovery compared favorably with a large control group of patients with delirium tremens who had received a liberal diet and all vitamin supplements.[1] Observations of this type have also been made in a group of ten patients with acute auditory hallucinosis. All of them recovered completely

while receiving a diet lacking in all vitamins, protein and fat. These results are shown in Table II.

The rate of recovery did not differ significantly from that observed in sixty-five patients with auditory hallucinosis who received a full diet and supplements of all B vitamins (fourteen recovered in less than one day, forty in one to six days, three in ten to twenty-five days and eight still had symptoms after more than six weeks.[2]

From these data, one may conclude that nutritional factors are not of primary importance in the causation of delirium tremens, acute auditory hallucinosis and related disorders. Although most of the patients eat virtually nothing during a spree, it is also a fact that in a significant number, symptoms develop on a background of adequate dietary intake. It would appear that spree drinkers replenish their nutritional stores between drinking bouts, to the extent that only a small proportion show the signs of nutritional disease. The observations that delirium tremens and acute auditory hallucinosis subside without the ingestion of solid food or vitamins would seem to negate all possibility of a nutritional factor playing a causative role in these syndromes.

The role of abstinence in alcoholic tremor, rum fits, transient hallucinosis and delirium tremens: The relationship of delirium tremens to abstinence, after established habituation to alcohol, rather than to the direct effects of prolonged intoxication, has been a subject of dispute for many years. In 1953 we reported our observations on the withdrawal phenomena, which seemed to be operative in all our cases in which reliable data could be obtained.[1] Briefly, it was pointed out that the mildest degree of this syndrome (tremor and nausea) may arise after only a few days of drinking, and becomes manifest after a short period of abstinence. The most severe form of the syndrome (delirium tremens) occurs only after a prolonged period of drinking and after several days of abstinence. A patient may show one or all of the symptoms under consideration but in the latter instance they tend to unfold in a predictable sequence after the patient is forced to abstain from alcohol, first tremulousness, then seizures and hallucinosis and finally delirium tremens. Recently, Isbell and his associates[4] have provided elegant confirmation of this withdrawal theory. These investigators succeeded in inducing all the symptoms under consideration in a series of volunteer subjects who had been deprived of alcohol after they had consumed about a pint of pure grain alcohol per day for periods up to eighty-seven days. The symptoms always began when the blood level of alcohol was rapidly and markedly reduced. One must postulate, therefore, that in the habituated patient the neurologic symptoms are the result of excessive and disorganized activity of those parts of the central nervous system normally acted upon by alcohol, after the diminution in the levels of blood alcohol, *viz.*, after sleep or enforced abstinence. Presumably the elevation of blood levels by oral ingestion or infusion of alcohol would suppress these symptoms but this aspect of the subject has not been studied systematically.

Diseases Associated with Protracted Alcoholism and Nutritional Depletion and Characterized by Specifically Localized, Bilaterally Symmetrical Neuropathologic Changes

We turn now to the third major group of illnesses associated with alcoholism. This comprises a relatively small but serious number of neurologic disorders: Wernicke's disease, Korsakoff's psychosis, polyneuropathy, retrobulbar neuropathy, the neurologic portions of the pellagra syndrome, alcoholic cerebellar degeneration, Marchiafava-Bignami disease and central pontine myelinolysis.

Wernicke's disease is characterized clinically by paralysis or weakness of eye movements, ataxia of gait and by mental disturbance. The eye signs consist of (1) nystagmus that is horizontal and frequently vertical, (2) paralysis of the external recti muscles and (3) paralyses of conjugate gaze, most often of horizontal gaze. Ptosis, pupillary abnormalities and internuclear ophthalmoplegia are occasionally encountered.

The ataxia of stance and gait, which is to be found in almost every patient except in those in whom a severe polyneuropathy precludes testing of this function, involves trunk muscles

and legs to a maximum degree. In contrast to the gross disorder of locomotion, a clear-cut intention tremor on finger-to-nose and heel-to-shin testing is infrequent. The ataxia is probably cerebellar in origin but is often mistakenly attributed to a peripheral neuropathy. Both the ophthalmoplegia and ataxia are transitory in nature, although they tend to recover incompletely. Most patients are left with a fine horizontal nystagmus, and a small proportion show a slight ataxia of gait, years after the onset of the illness.

A mental disorder was present in 90 per cent of our patients and the psychic abnormality proved the most difficult to define and to measure. At least three different types could be recognized.

(1) About 20 per cent of patients showed the characteristic mental symptoms of delirium tremens or its variants. These symptoms were present at the beginning of the illness, were evanescent and cleared without any specific treatment. We assumed because of their low incidence and their known behavior in the withdrawal syndrome, that they were not part of the Wernicke syndrome.

(2) The majority of patients, when first seen, were in a state of apathy and profound confusion. Unconsciousness as part of the acute phase of the disease or at any other time was very rare. Even drowsiness was not common; instead the patient's state tended to be one of disinterest and indifference. Under the influence of adequate diet, or of thiamine alone, the patient rapidly became more alert, attentive and responsive and more capable of partaking in mental testing. Then the most prominent abnormality was one of retentive memory, which is the cardinal feature of *Korsakoff's psychosis*.

(3) The most prominent and constant mental abnormality, from the beginning, was in the sphere of memory and learning and is generally referred to as Korsakoff's psychosis. This disorder, as was already stated, tends to emerge as the *early global confusion* subsides but probably was present, although masked, from the beginning. Usually it developed in a person who initially exhibited ocular palsy, nystagmus or ataxia, although it may appear to have developed insidiously and be unaccompanied by these other neurologic disturbances. The state generally defined as Korsakoff's psychosis emphasizes the "memory defect with confabulation." Such a designation does not, however, adequately characterize this psychosis, insofar as our patients also showed a number of abnormalities in cognitive function which depend little or not at all upon memory. The administration of the Wechsler-Bellevue Intelligence Scale showed them to be defective in tests designed to measure concentration, capacity to form verbal and visual abstractions, the ability to shift from one mental set to another and to learn in a new situation.[5] The more detailed psychologic tests devised by Talland[6,7] have convincingly demonstrated an impairment of perceptual function and of concept formation.

Korsakoff's psychosis is unique not only in respect to these psychic defects but also in the fact that alertness, attentiveness and capacity to submit to a psychological test are all relatively intact. Memory function is regularly impaired out of all proportion to other cognitive functions. Thus, a patient may, within a few minutes of taking and performing well on an intelligence test, be unable to recall the examiner or having taken the test. In addition, there is an extensive retrograde amnesia which may cover a variable period of months or even years. It is the recent memory that suffers most although this is a relative matter, and remote memories are also lost to a varying degree.

Another aspect of the memory disorder that is particularly noteworthy is the persistent inability to learn newly presented material, i.e., to establish "new memories." Since the adaptation to every new situation requires the forming of new memories, or at least combining new and old ones, it is this defect which renders the patient helpless in society and incapable of performing any but the most routine tasks. Another feature of the condition is the derangement of the perception of time. Past events cannot be recalled in proper sequence or localized temporally. This defect becomes obvious after the acute state of the illness has passed and some improvement in memory function has occurred, and it remains the pre-eminent ab-

normality in all but the few patients who made a complete recovery.

Confabulation, often regarded as essential for the diagnosis, is another of the more dramatic symptoms. Here one has reference to the patient's tendency to relate some experiences of the distant past as having happened recently or of actually fabricating a story which is totally fictitious. This phenomenon was often present but was by no means specific, occurring as it did in many other confusional and dementing states. The diagnosis of Korsakoff's psychosis can be readily made without it. Confabulation tends to disappear with time and is an exceptional finding in the chronic stage of the illness.

Thus in the study of the natural course of Wernicke's disease it was noted that the mental symptoms, like the ophthalmoplegic and ataxic ones, are not uniform or static, but present in a regularly changing sequence. Originally a quiet confusional state, characteristic of acute Wernicke's disease, and less frequently an associated delirium, dominates the scene. Later, an amnestic-confabulatory state, usually designated as Korsakoff's psychosis, becomes prominent. Finally, varying degrees of memory defect without confabulation or confusion (except as relates to poor memory), frequently referred to as the alcoholic deteriorated state, form the chief aberration. The series of laboratory observations which we have made on this group of patients is summarized in the reports of Talland.[5–11]

It is evident, even from this brief description, that any distinction between Wernicke's disease and Korsakoff's psychosis, such as has been made in the past, may be artificial. In support of the hypothesis that Korsakoff's psychosis is but the psychic component of Wernicke's disease, we have assembled the following lines of evidence.

(1) In sixty-two of seventy-two patients with Wernicke's disease, who presented with ocular palsy, ataxia and confusion, and who survived for longer than a few days, the characteristic amnestic disorder of Korsakoff's psychosis became evident.[1] Conversely, in two large series of patients with Korsakoff's psychosis who were examined in the Boston and Worcester State Hospitals, the majority showed the stigmata of Wernicke's disease, i.e., nystagmus and occasionally mild ataxia, even years after the onset of their illness.[12,13]

(2) Pathologically also there is indication of the unity of the two diseases. Except for differences attributable to the age of the lesions, the pathologic changes were the same whether the patient died in the acute stages of the disease or in the chronic phase, after the ocular palsies had cleared and the amnestic symptoms dominated the scene. In a series of twenty-three patients diagnosed during life as having Korsakoff's psychosis all showed the typical pathologic findings of Wernicke's disease. Conversely, all our patients with pathologic evidence of Wernicke's disease (now fifty-one in number) had had the symptoms of Korsakoff's psychosis, when the history and examination were adequate to assess this fact.[14] Therefore, we have assumed for purposes of our study that we are dealing with a single disease entity.

In these illnesses, as in delirium tremens and related disorders, three further types of data were secured: (1) the dietary history and nutritional status on examination, (2) direct observations of the symptoms and signs under conditions of dietary control and (3) the effect of alcohol.

(1) The dietary history and nutritional status were determined in a group of 186 patients with the acute manifestations of Wernicke's disease. The precise dietary aberration in these patients was difficult to determine. However, in every case in which a reliable history could be obtained, an over-all reduction in food intake had occurred, often astounding in degree. It was characteristic, for instance, to be told that the patient had not eaten an adequate meal for a year's time, or that he would not take a morsel of food for several days on end, or only a few spoonfuls of soup or half a sandwich. In general, the alcoholic and dietary habits of this group were readily distinguished from those of the spree drinker. The latter type drinks excessively for a circumscribed period, during which time he eats little or nothing. Between sprees, however, he usually eats well and quickly regains a normal nutritional state. In patients with Wernicke's disease and Korsakoff's psychosis, the daily imbibition and state of

semistarvation is to be measured in months and often years.

In 170 of these 186 patients examination disclosed clear evidence of nutritional depletion, as manifested by marked generalized thinness, redness and depappilation of the tongue and dryness and coarseness of skin. In addition, fifty-one patients had signs of advanced liver disease, and in twenty-four of these, cirrhosis was a primary or contributing cause of death. Eleven patients had advanced pulmonary tuberculosis, and the occurrence of this disease was probably influenced by their poor nutritional status.

(2) The second portion of the study was undertaken to define the effect of specific nutrients on the individual clinical signs of Wernicke's disease and Korsakoff's psychosis. Experimental observations have now been made on fifty-one patients. Twenty-nine of these, whose recovery was closely observed while they were receiving a full diet and all vitamin supplements, served as controls. In general, the procedure was similar to the one already described in an earlier report.[15] The patients received no food from the time they were admitted to the hospital until their symptoms had been assessed and quantitated. The power of abduction of the eyes was used as an index of the degree of ophthalmoplegia. A total inability to abduct the eye beyond the midline was considered as 4 plus, a slight abductive movement as 3 plus, moderate abduction as 2 plus and almost full lateral movement with only a slight disalignment of eyes, as judged by the small amount of sclera showing between the limbus and outer canthus or any inability to sustain lateral gaze for more than a few seconds, as 1 plus. Coarse, well sustained nystagmus on lateral or vertical gaze was designated as 4 plus, lesser degrees of it as 3 or 2 plus and a barely perceptible nystagmus as 1 plus. With respect to ataxia, 4 plus denoted an inability to walk even with some assistance; 3 plus meant that the patient could walk if aided; 2 plus indicated ability to walk alone but with staggering; and 1 plus represented competent locomotion but with slight ataxia. The mental symptoms were difficult to quantitate, and we were content to simply divide them into the categories that have already been described; i.e., mild delirium, a global confusional-apathetic state, the typical Korsakoff amnestic-confabulatory syndrome and the final alcoholic pseudedomentia.

The experimental patients were then given one of three dietary regimens: a solution containing 200 gm. glucose and 1.3 gm. sodium chloride per liter of water;[15] the rice diet of Kempner;* the vitamin B-deficient diet of Williams et al.[16] To each of these deficient diets, different B vitamins were added, in amounts and at intervals specified herein. A special group of four patients were given liberal doses of whiskey in addition to the deficient diet and vitamins. They were compared with the control subjects and others on the vitamin B deficient diets who were forced to abstain completely.

Clinical experiments of this type were found to be difficult to carry out in a general hospital and our facilities were crude, to say the least. The cooperation and vigilance of the house staff and nurses was difficult to maintain. Also, the administration of a grossly deficient diet to a nutritionally depleted patient raised special problems. A diet consisting of glucose, saline and other minerals and water can be tolerated for only a few days at most because it deprives the patient not only of vitamins but also of protein and fat. Furthermore, we could not in all good conscience offer even a slightly unbalanced diet in the face of a nutritional disorder for more than a few hours or days (even though it did conform to the diet on which the patient had been living) if there appeared to be a serious hazard of augmenting the damage to the central nervous system.

We can report that each patient was followed so closely that the nutritional study could be terminated at the earliest sign of progression of the neurologic syndrome. None of the deaths occurred in patients receiving a purified diet and to the best of our knowledge not one of the experimental subjects was permanently harmed.

These remarks are offered by way of preliminary apology for the obvious imperfections of some of our clinical studies. Nevertheless, it

* See footnote to Table 1.

will be seen that at least some of the results permit firm conclusions to be drawn. Others, it must be conceded, are so indecisive as to indicate the need of further study under more carefully controlled conditions.

The results of these clinical-experimental studies may be summarized as follows: In our original study, the results of which have been published,[15] nine patients with Wernicke's disease were given a synthetic diet composed only of glucose, minerals and water. Specific vitamins were added after appropriate intervals of observation. Prior to the administration of thiamine there was no improvement in any of the signs. More specifically, despite alcohol withdrawal, bed rest and the addition of other vitamins (exclusive of thiamine) the ophthalmoplegia progressed, while the nystagmus decreased only in association with an increase in ocular paralysis. When thiamine alone was added to the purified diet, the ophthalmoplegia began to improve within a few hours, and cleared completely within a few days to a week. Diminution in nystagmus and ataxia also occurred, but the change was more gradual and these symptoms persisted in mild form for months or years after their onset. The effect on the mental disturbance was difficult to judge and was minimal in degree; it was thought that the patients showed an increased attentiveness and capacity to maintain a conversation and a greater ease of confabulation.

Because of the restrictions imposed by the use of the glucose diet, the second experimental group, comprising twenty-seven patients, was given the Kempner rice diet. When first examined, all of them had ophthalmoplegia and nystagmus, twenty-three had ataxia and twenty-two had a profound confusional-apathetic state. The patients were observed for twelve to twenty-four hours before any vitamins were added. During this period the symptoms either remained stationary or became somewhat worse. When thiamine alone was added to the diet, a clearly beneficial effect on the opthalmoplegia, nystagmus and the ataxia was noted, similar to that recorded in the previous experiment. The symptoms of apathy, drowsiness, listlessness, inattentiveness and inability to concentrate and to sustain a conversation cleared rapidly after the administration of thiamine alone. The latter findings confirmed the impression obtained from the original experiment using the glucose diet.

Four patients were given alcohol (45 ml. of 90 proof whiskey every four hours in water) for periods of seven to ten days, in addition to the rice diet and thiamine. Their ophthalmoplegia and ataxia improved at the same rate as they did in the larger control group who received only a nutritious diet, or in the group who received only the rice diet and thiamine.

With respect to memory defect and confabulation, i.e., Korsakoff's psychosis, no significant improvement could be discerned within the period of glucose and thiamine administration, which measured eleven days in the longest instance, or in the period covered by the administration of the rice diet and thiamine, which measured fourteen days at most. Drawing an analogy to the slow rate of recovery of peripheral neuropathy, it was believed that these periods may have been too short to evaluate the effect of thiamine. Accordingly, observations were made on a group of twelve patients who were maintained on a special vitamin B-deficient diet[16] for periods as long as eight weeks, thiamine being the only nutritional supplement. In four of these patients the memory defect remained unchanged, in two there was a complete recovery, in six others there was a partial recovery. This outcome was more or less similar to that which occurred in a control group of twenty-nine patients who who had been given a full diet with all the vitamin supplements from the onset of their illness. The mode of recovery in the experimental and control patients is shown in Table III.

Several other isolated observations may be important. Three patients have been studied who, at the time of admission to the hospital, showed only ophthalmoplegia and ataxia, but no mental abnormalities. One of these patients was given only the glucose and water diet. His ophthalmoplegia and ataxia became progressively worse and on the third hospital day he was noted to be severely confused, disoriented in time and place, unable to give a clear account of any of his symptoms or remember

TABLE III
Relation of Nutritional Factors to Recovery from Korsakoff's Psychosis
(Period of Observation on Controlled Diets)

Diet	Full Recovery	Partial Improvement	Little or No Improvement
Deficient diet + thiamine (N = 12)	2 (2–3 wk.)	6 (1–8 wk.)	4 (2–8 wk.)
Full diet + all vitamins (N = 29)	3 (2 wk.) 4 (8 wk.) 7 (2–24 mo.)	5 (2–8 wk.) 8 (3–18 mo.)	2

anything that was said to him, and confabulated at the slightest provocation. This state persisted for twenty-four hours, after which he was given a full diet and all vitamins. The general confusion and confabulation cleared in several weeks; the amnestic symptoms reverted slowly but completely over a three-month period.

The second patient had been abstinent for five days before admission. He was given a rice diet with no vitamins. Four days after admission, a confusional state developed, characterized mainly by a disorientation in place. This persisted for thirty minutes, at which time he was given 100 mg. of thiamine intramuscularly. He continued to speak in a confused manner for another two hours, after which he fell asleep. The next morning his eye signs were considerably improved, and no mental abnormality could be detected. For the next seven days, the patient was maintained on the rice diet, thiamine (50 mg. daily) being the only nutritional supplement; during this time the ophthalmoplegia and ataxia cleared completely, and his mental state remained intact.

The third patient, who also showed ophthalmoplegia and ataxia without mental signs, was given a rice diet from the time he entered hospital but this was supplemented by 50 mg. thiamine given intramuscularly each day. He was carefully observed for a fourteen-day period, during which time he made a complete recovery from the ophthalmoplegia and ataxia, without ever having any mental abnormalities.

In view of these observations, there seems little doubt that the ophthalmoplegia, nystagmus and ataxia of Wernicke's disease are related to thiamine deficiency. The marked sensitivity of the ophthalmoplegia to the administration of thiamine accounts for the rapid disappearance of this sign following a meal or two; the quality of prompt reversibility suggests that these symptoms are due to a biochemical abnormality which has stopped short of maximal structural change.

The relation of thiamine administration to the clearing of mental symptoms is a more difficult problem to assess. In the experimental studies described, symptoms of apathy and drowsiness became strikingly worse when thiamine was withheld and cleared rapidly after the administration of thiamine. Isolated observations also suggest that symptoms such as these may develop if the patient is denied thiamine, and that a patient with ophthalmoplegia and ataxia can be protected from having these mental symptoms by the administration of thiamine alone. It is very likely, therefore, that this aspect of the mental disorder is related to thiamine deficiency.

Recovery from the amnestic symptoms is slow and often incomplete. The most likely explanation of this imperfect recovery is that the memory loss, once fully developed, depends on a structural rather than a biochemical lesion. The fact that lesions of the mammillary bodies and thalamus are consistent pathologic findings in patients with Korsakoff's psychosis would tend to substantiate this view.

It is of interest that the rate and degree of recovery from the learning and memory defects was roughly parallel in our experimental and control patients. The relative failure of

the amnestic symptoms to respond either to thiamine or to all the nutritional supplements may be governed by the intensity and extensity of the diencephalic lesions and the inherent slowness of recovery of damaged brain tissue. It is still possible that the amnestic symptoms depend on thiamine deficiency and need not be due to a deficiency of several vitamins, as has been suggested by some authors.[17] From our studies, as from others (of nonalcoholic cases in prisoner of war camps), there is no evidence that alcohol is directly responsible for this neurologic condition or that abstinence plays any part in it.

Alcoholic polyneuropathy: The clinical features of this disease are well known, and need not be elaborated here. That alcoholism and polyneuropathy are closely associated has long been appreciated.[18,19] However, the concept that this polyneuropathy has a nutritional origin is relatively recent. Only after beriberi became established as a nutritional disorder was the alcoholic etiology questioned. The similarity of neuritic beriberi to alcoholic neuritis was commented on by several authors, but it was Shattuck[20] in 1928, who first clearly perceived their relationship. He suggested that the "polyneuritis of chronic alcoholism was caused chiefly by failure to take or assimilate food containing a sufficient quantity of vitamin B and might properly be regarded as true beriberi." Convincing evidence that alcoholic polyneuritis does not result from the neurotoxic effect of alcohol was supplied by Strauss.[21] Ten patients who continued their daily consumption of whiskey while eating a well balanced diet supplemented with yeast and vitamin B concentrates made an adequate recovery.

We have not repeated Strauss's experiment but have done the converse, i.e., observed the course of the clinical symptoms in twelve patients with alcoholic polyneuropathy who were deprived of alcohol and given only a vitamin B-free diet. Although the period of observation on this regimen was very short (five days at the most) it was obvious that the neuritic symptoms and signs did not improve in any of the patients and that they worsened perceptibly in most of them. With the addition of thiamine alone to the vitamin B-deficient diet there was improvement in all cases. In ten patients the improvement was purely symptomatic with no measurable effect on the neuritic signs; however the period of observation after the addition of thiamine was only two weeks in the longest instance. In two patients, who were maintained on a vitamin B-deficient diet plus thiamine alone for eight full weeks, a definite improvement occurred in motor and sensory signs and there was a return of ankle jerks, where previously they had been absent. Interestingly four of these ten patients suffered a worsening of symptoms and signs for a period of two to three days after the addition of thiamine, following which they also improved symptomatically. We believe that we are justified in the conclusion, therefore, that alcoholic polyneuropathy is a nutritional problem and not an alcoholic one *per se*.

The precise nutritional factors concerned in the neuropathy of alcoholism and beriberi are not yet fully defined. For a number of years after the discovery of thiamine, a debate centered around the question of whether this was the antineuritic vitamin.[22,23] Only a few of the experiments undertaken to settle this point satisfy the strict criteria of the nutritionist; however, there is a small but acceptable body of evidence that in the rat, dog and pigeon thiamine deficiency brings about a degeneration of the peripheral nerves.[24–26] The necessity of either accepting or rejecting the specificity of thiamine became less urgent when it was shown that a deficiency of pyridoxine or of pantothenic acid in swine caused degeneration of the peripheral nerves.[27,28] In man, also, there are isolated instances in which neuropathy has been produced by a deficiency of thiamine,[16,29,30] pyridoxine[31] or pantothenic acid.[32]

Alcohol amblyopia, or tobacco-alcohol amblyopia, refers to a disorder of vision in alcoholic subjects, characterized clinically by the presence of central or centrocecal scotomas (more prominent with red and green than with white test objects). Few pathologic studies of it have been made. In one fatal case we observed a degeneration of fibers in each of the optic nerves, chiasm and tracts, corresponding in location to the papillomacular bundles. It

TABLE IV
Symptoms and Signs of Nutritional Deficiency in Fourteen Patients with Alcohol Amblyopia

Data	No. of Patients
Inadequate diet for months or years	14
Extreme weight loss just prior to onset of visual symptoms	8
Signs of nutritional deficiency (thinness, tongue and skin changes)	14
Polyneuropathy	11
Wernicke-Korsakoff syndrome	5
Spinal spastic ataxia	2

is a relatively rare condition in the population of alcoholics whom we studied, occurring about once in every twenty-five patients with polyneuropathy. Recently we have reported our observations on this illness, based on a study of fourteen patients.[33] The nutritional data are summarized in Table IV.

We have been unable to study these patients under controlled dietary intake or to investigate the role of alcohol. However, Carroll[34–36] has shown that recovery from tobacco-alcohol amblyopia occurred when patients were given their usual intake of alcohol and tobacco, providing they took a nutritious diet or a deficient diet and B vitamins. Moreover, the so-called alcohol-tobacco amblyopia is clinically and pathologically indistinguishable from the nutritional amblyopia observed in prisoner of war camps.[37,38] The conclusion is justified that this represents a nutritional problem related to a deficiency of B vitamins and not to alcohol *per se*. However, the specific nutrient responsible is uncertain. Animal experiments have not solved the problem. Isolated reports have implicated riboflavin,[38] vitamin B_{12}[39] and vitamin B_1,[40] but the evidence in each of these reports is far from conclusive.

Pellagra: In the United States the incidence of pellagra has decreased sharply in the past decade, not only in the alcoholic population in northern municipalities but also as an endemic dietary problem in the south. In a personally examined series of 131 alcoholic patients with nutritional disorders of the nervous system only one had pellagra.[1] The virtual abolition of this disease in the alcoholic population has been the subject of a recent study by Figueroa et al.[41] who attribute this phenomenon to the enrichment of bread with niacin.

The neurologic disorder which accompanies pellagra can be referred to all parts of the nervous system. In the early stages of the disease, along with the diarrhea and changes in skin and mucous membranes, mild psychic abnormalities, which may be mistaken for those of psychoneurosis, occur. In the more advanced stages of the disease there is a confusional psychosis which, in more chronic forms, resembles dementia. Symptoms relating to disease of the posterior and lateral columns of the spinal cord accompany the severe psychic alteration. Often there is an associated polyneuropathy indistinguishable from alcoholic or endemic beriberi.

We have had no opportunity to study the role of nutrition and alcohol in this disease and can only refer to the work of Ruffin and Smith,[42] Sydenstricker and Thomas[43] and Spies and DeWolf.[44] The latter, in 1933, were the first to discredit the entity of alcoholic pellagra by demonstrating that alcoholic patients recovered from pellagra, despite the daily intake of large amounts of corn whiskey, provided that they ate an adequate diet supplemented with yeast. Their observations further suggested that alcoholic and endemic pellagra were identical and that the relationship between the two was one of substitution of drink for food.

The subject of the nutritional defect in pellagra is highly complicated. Nicotinic acid deficiency represents only one aspect of the matter. The amino acid tryptophan serves as a precursor of nicotinic acid and it has been discovered that an adequate amount of tryptophan is necessary to prevent pellagra if the diet is limited in nicotinic acid. The relationship between niacin and its precursor tryptophan, as well as between the many ancillary factors concerned with the etiology of pellagra, has been well discussed by Goldsmith and her collaborators.[45] Nicotinic acid deficiency may possibly account for the encephalopathy of pellagra, but the other neurologic manifestations, e.g., polyneuropathy, are probably related to the deficiency of B vitamins other than niacin.

Cerebellar cortical degeneration: This refers to a cerebellar syndrome in alcoholic patients, characterized by an ataxia of gait and of the legs, with relatively little involvement of the arms, speech and ocular motility and, in the majority of cases, by an evolution over a short period of time followed by years of stability. Pathologically, there is a degeneration, varying in severity, of all neurocellular elements of the cerebellar cortex, particularly the Purkinje cells, with a striking topographic restriction to the anterior and superior aspects of the vermis and of the hemispheres.

Our own experience with this disease is based on the study of fifty clinical and eleven postmortem cases.[46] Unfortunately, this relatively large number of cases has not been well suited to the clinical investigation of causative factors, for in nearly all instances our first contact with the patient occurred long after the inception of the disease. We had only retrospective data and the hospital records from which to draw conclusions, and these are notoriously inaccurate. All our patients had been inveterate alcoholics, but it is noteworthy that six of them claimed to have abstained from alcohol for varying periods of time before the onset of their cerebellar symptoms. One must, therefore, reserve judgment on the toxic effect of alcohol as the direct causative agent.

There was evidence from the history and examination that a serious disturbance of nutrition had occurred in the majority of the patients. The pertinent nutritional data is indicated in Table v.

TABLE V

Symptoms and Signs of Nutritional Deficiency in Fifty Patients with Alcoholic Cerebellar Degeneration

Data	No. of Patients
Inadequate diet for months or years	39
Extreme weight loss just before onset of cerebellar disease	8
Signs of nutritional deficiency (thinness, tongue and skin changes)	29
Polyneuropathy (mild)	23
Wernicke's disease	5
Amblyopia	2
Cirrhosis of the liver	14

It will be seen that eleven patients showed no signs of nutritional abnormality when they were first seen by us, nor did they give a history of inadequate diet. This does not necessarily mean that malnutrition was not a factor in these patients, since they sought medical help long after (six months to thirteen years) the onset of their illness, at which time their alcoholic and perhaps their dietary habits had changed considerably.

A number of reports in the medical literature may have a bearing on the nutritional aspect of the etiology. Stannus[47] described a child with recurrent pellagra who also showed a fluctuating cerebellar syndrome. Ransome,[48] commenting on this report, quoted his experience with a series of eighteen cases of pellagra which occurred in Singapore in 1940–1941, many of which showed a gross cerebellar syndrome. Danaraj[49] described an elderly Chinese man in whom progressive cerebellar signs developed on a background of severe nutritional deficiency; the neurologic signs disappeared within three months after the institution of a liberal diet plus nicotinic acid and riboflavin. In the patient of Houssiau[50] a transient cerebellar syndrome developed following a period of severe vomiting and weight loss associated with amebiasis. All these cases are acceptable clinical examples of cerebellar disease, particularly since there were no signs of polyneuropathy or posterior column affection in any of them. However, in none of these cases was the lesion verified pathologically.

There are remarkably few accounts of nutritionally-induced changes of the cerebellum in the experimental animal. Miyagawa[51] and Swank and Prados[52] reported fragmentation and irregular thickening of fiber systems of the cerebellar cortex in thiamine-deficient pigeons, but such findings in themselves can hardly be considered significant. Crude cerebellar lesions, consisting of edema, necrosis and hemorrhage have been produced in chicks by the administration of a diet consisting of milk powder, starch, cod liver oil, salts, filter paper and dried baker's yeast.[53] Although these lesions probably resulted from nutritional deficiency, it does not appear justified to attribute them to a specific lack of vitamin E. Lher-

mitte, de Ajuriaguerra and Garnier[54] described the occurrence of chromatolysis and extreme pallor of the Purkinje cells of rabbits who were fed alcohol for a period of several weeks; these changes were reportedly not seen in animals who had received supplemental thiamine. This work suffers from the fact that the lesions were not illustrated; also the changes described in the Purkinje cells were entirely qualitative ones, and must therefore be interpreted with caution.

Marchiafava-Bignami disease: This rare complication of alcoholism, originally described in Italian males addicted to crude red wine, is now known to occur in nonItalians, and not exclusively in wine drinkers. The symptomatology is diverse and includes disorders of cognitive function and emotional control, delirium, convulsive seizures, varying degrees of tremor, rigidity and paralysis, and ultimately, coma. Pathologically the disease is characterized by a symmetrical degeneration of myelin and, to a lesser extent, of axis cylinders, sometimes progressing to cavitation. The corpus callosum is most frequently affected, and less often the central parts of the anterior commissure, the central and convolutional cerebral white matter, the optic chiasm and radiations, and the middle cerebellar peduncles. The predilection of this disease for commissural fiber systems has been stressed, but this claim may not be completely justified, since it is not confined to these fibers.

Inasmuch as these cases usually occur on a background of chronic alcoholism, a direct toxic effect has generally been accepted as the cause. In support of this idea the work of Testa[55] is frequently cited in which lesions topographically similar to those of Marchiafava-Bignami disease were reportedly produced in dogs by the chronic ingestion of alcohol. However, the pathologic descriptions are far from satisfactory, and Testa's findings have not been confirmed by others. In the brains of cats which had been fed large amounts of alcohol for periods as long as two years we were unable to detect any abnormalities of this type, at least in sections stained by the methods of Nissl and Loyez.[56] On the other hand, there are frequent allusions to states of malnutrition in these patients. Furthermore, cases have been verified pathologically[57,58] in which there was no history of alcoholism. An additional point in favor of a possible nutritional cause is the conjunction of Marchiafava-Bignami disease and other proved deficiency diseases of the nervous system, particularly Wernicke's disease.[58–63]

Central pontine myelinolysis: Under this heading we have described a unique disease of the central nervous system in which the myelin sheaths of all the nerve fibers in the central part of the basis pontis had been destroyed in a single, large, symmetrical focus. The nerve cells and axis cylinders were largely spared, and the blood vessels were patent and unaffected. There were no signs of inflammation in or near the lesion.[64]

In three of our cases there was a well documented history of prolonged and serious alcoholism and of malnutrition of varying severity. The fourth case developed on a background of severe malnutrition in a patient who had never touched alcohol. Since our original report, we have encountered several other instances of this disease in patients who also showed the lesions of Wernicke's disease[14]; one such instance has recently been reported from France.[65] Similar cases have been discovered in midwestern United States[66] and Canada[67] in nonalcoholics. It may be concluded that alcohol is not the important factor in this disease. One must consider the possibility of a nutritional etiology. Clinically, this appeared to be a factor, in our cases at least. Pathologically, the singular features of this disease are the symmetry of the lesions and the constancy of their localization. These attributes must be meaningful, insofar as they are common to the diseases of known nutritional or metabolic origin. Obviously more data are needed before a final statement can be made regarding the role of nutrition in this disease.

Disorders of Nervous Function in the Patient with Alcoholic Cirrhosis

A wide variety of neurologic symptoms are known to accompany all types of liver disease; and, since a significant number of alcoholic patients suffer from hepatosis and cirrhosis, it

is not surprising that these nervous symptoms should appear with great frequency in the alcoholic population. A disorder of consciousness, taking the form of confusion, stupor or coma and developing over a few days, represents the central nervous abnormality. This may be accompanied by an inability to maintain the limbs in a stable posture (called asterixis by Adams and Foley[68]) with a resultant "flapping" tremor of the outstretched arms, and less often by choreoathetotic postures or cerebellar dysarthria and ataxia. The symptoms may be evanescent and may recur; or, once started, the clinical state may progress to death within a few days. In a small number of patients with chronic liver disease, there may occur chronic cerebellar or extrapyramidal symptoms. These cases resemble Wilson's disease but lack its relatively specific defect in ceruloplasmin and disordered copper metabolism. Thus, two syndromes, an acute fatal or episodic one with predominant disturbance of consciousness, and a subacute or chronic one with predominant cerebellar-extrapyramidal disorder, have emerged.

These conditions, which can usually be distinguished on clinical and pathologic grounds from the aforementioned alcoholic diseases, do not appear to bear a direct relationship either to alcoholic intoxication (or withdrawal) or to a dietary vitamin B deficiency. The same disorders occur in nonalcoholic liver disease; and although alcoholic cirrhosis and coma are complications and a frequent cause of death in the depleted alcoholic, the same syndromes have been observed in alcoholics who had been receiving a nutritious diet. Thus far, a single biochemical mechanism underlying the encephalopathy has not been isolated. A considerable number of cases of hepatic coma have high levels of blood ammonia and this in some manner interferes with the metabolism of the brain. In others, sodium, chloride or potassium depletion or abnormality in carbohydrate metabolism have been incriminated. In the few remaining cases there is no satisfactory biochemical explanation at the present time.

In conclusion, one can say that the liver and the multitude of metabolic reactions that it governs may play a part in many diseases now considered nutritional. It probably has a relationship to several of the alcoholic diseases considered here, but much work remains to be carried out before these relationships can be defined.

SUMMARY AND CONCLUSION

The neurologic disorders of the alcoholic patient have been considered with special reference to the causative role of alcohol, nutritional and other factors. The results of our investigations and of others warrant the delineation of a large group of diseases known as delirium tremens and its variants, alcoholic epilepsy and alcoholic auditory hallucinosis, as being causally related to habituation and withdrawal from alcohol. These diseases are *not* of nutritional origin.

In contrast, Wernicke's disease, alcoholic Korsakoff's psychosis, polyneuropathy, retrobulbar neuropathy and the neurologic manifestations of pellagra are clearly nutritional diseases related to alcoholism only through the dietary irregularities to which it gives rise. The pathogenesis of alcoholic cerebellar degeneration, central pontine myelinolysis and Marchiafava-Bignami disease have yet to be investigated and their nutritional status remains uncertain.

On the basis of these observations the following classification of the alcoholic neurologic disorders is proposed: (1) alcoholic intoxication—drunkenness, combativeness (pathologic intoxication), coma; (2) the abstinence or withdrawal syndrome: tremulousness, "run fits," hallucinosis, delirium tremens and its variants; (3) nutritional disorders of the nervous system secondary to alcoholism: the Wernicke-Korsakoff syndrome, polyneuropathy, amblyopia, pellagra; (4) diseases of uncertain etiology: cerebellar degeneration, Marchiafava-Bignami disease, central pontine myelinolysis; and (5) disorders of nervous function due to alcoholic cirrhosis.

The studies of the nutritional aspects of Wernicke's disease further demonstrate that the ophthalmoplegia, ataxia and the nystagmus are due to a specific lack of thiamine. Certain of the mental symptoms, such as apathy and drowsiness, also respond dramatically to the

administration of thiamine alone. The amnestic symptoms respond more slowly and incompletely, but evidence has been presented that they also may be related to thiamine deficiency.

One interesting and practical conclusion of our studies has been that Korsakoff's psychosis and alcoholic dementia or pseudoparesis are the common psychic manifestations of Wernicke's disease. They may, therefore, be attributable (like Wernicke's disease of which they are a part) to nutritional deficiency, more specifically to a deficiency of thiamine. This would mean that one of the most frequent and serious sequelae of chronic alcoholism results simply from the substitution of alcoholic beverage for nutritious food. This problem demands our most thoughtful attention, not only because of its prevalence and seriousness, but also because it is potentially preventible.

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DISCUSSION

Dr. Joseph M. Foley (*Jersey City, New Jersey*): Dr. Victor has given a classification of the neurologic diseases which are the result of indulgences in alcohol. He agrees that much work remains to be carried out to clarify some of these states. I think he also will admit that there is a basis for substantial disagreement on some of the conclusions he has reached.

Dr. Davidson, would you like to comment on the overlap of the brain and the liver?

Dr. Charles S. Davidson (*Boston, Massachusetts*): I would like to ask Dr. Victor one question. With regard to the evidence that polyneuropathy in alcoholics is due to nutritional deficiencies and vitamin B_1 deficiency, as I understand it, there is still some argument about this among the experts and I wonder if this has been proved beyond a doubt.

Dr. Foley: Are there any other questions? If not, I would like to make one or two observations, and perhaps suggestions, that stem almost automatically from what Dr. Victor has had to say.

I have some strong beliefs about the occurrence of Wernicke's encephalopathy, and one of the things that is striking in a hospital situation is the frequency with which Wernicke's disease develops after the patient is admitted to the hospital.

It is almost impossible, I should think, nowadays, to get into a hospital for any cause (even as a visitor, sometimes), without getting an infusion of glucose. With alcoholics we run into the situation in which the combination of alcohol withdrawal and increased metabolic demand is inflicted upon the patient at the same time. The withdrawal symptoms of themselves constitute an excessive metabolic demand. Therefore, I wonder if Dr. Victor would approve of the practice that is now exercised in many places: starting the patients on thiamine immediately upon admission to the hospital.

I should like to ask about the matter of the withdrawal seizure. Is there any evidence that anticonvulsants administered at the time of withdrawal can in fact prevent the seizures?

One other point that strikes me is the myoclonic feature of the alcoholic withdrawal seizure. These patients so often seem to be very sensitive to stimulus, and ultimately it would seem the twitches of delirium tremens merge into a fit under the stimulus of a loud noise or a startling psychologic situation.

Dr. Davidson: I would like to ask one more question, would Dr. Victor comment on the possibility that the fits in the alcoholics might be a deficiency of vitamin B_6?

Dr. Maurice Victor (*Boston, Massachusetts*): The first question, whether polyneuropathy is purely of nutritional origin or whether alcohol plays a part in its genesis will, I assume, be considered in detail by Dr. Hornabrook. I will only say that I know of no good evidence that polyneuropathy occurs in well nourished alcoholics. Furthermore, there appear to be no significant differences between neuritic beriberi, in which alcohol plays no part, and the neuropathy in alcoholics seen in the Occident. The most telling evidence in favor of a nutritional origin is that of Strauss: ten patients with alcoholic polyneuropathy were allowed to drink in their usual fashion provided they took an adequate diet; all of them made a recovery that was comparable to the recovery made by patients who had been given a good diet alone.

I agree entirely with Dr. Foley in regard to the therapy of alcoholism and particularly of Wernicke's disease. Every alcoholic patient should be treated with a good diet and vitamin supplements because the nutritional history is often lacking or unreliable and we cannot with certainty decide which alcoholic patient is nutritionally depleted.

Whether or not anticonvulsants should be used in the withdrawal state in order to prevent seizures is a statistical problem for which no solution is yet available. I can say that seizures in this setting occur over such a circumscribed period of time, and so early in the phase of abstinence, that most of our patients have finished with their seizures before anticonvulsant medication would be effective. Furthermore, the prevention of future seizures depends upon abstinence from alcohol rather than on anticonvulsants. If the patient starts to drink again, he usually forgets to take his anticonvulsants; if he does not drink, he does not need the anticonvulsants.

Whether or not vitamin B_6 deficiency plays a role in the etiology of "rum fits" is a moot question. I believe Dr. Davidson carried out some investigations on this problem and believes that it does play a role.

DR. DAVIDSON: Dr. Lerner and I did perform some work on this.

DR. VICTOR: Although we do not have any direct evidence that would contradict your contention, I would make the following critical comments: it has been pointed out that the majority of patients who present with seizures and other manifestations of abstinence have been eating poorly for variable periods of time. It has been our experience that most of these patients are depleted of sodium, potassium, chloride and magnesium, and that some of them are depleted of essential vitamins as well. We regard the depletion of these substances as associated rather than causative factors in the abstinence syndrome. Possibly pyridoxine partakes of this general depletion and should be similarly regarded. In regard to Dr. Foley's last questions, I assume that he is referring to the contention that, because of chronic gastrointestinal disturbances, alcoholics do not absorb nutrients properly. Although this statement is made repeatedly, I know of no good data to support it. My own belief is that with isolated exceptions, as with persistent vomiting or diarrhea, the nutritional defect in alcoholics is the result of inadequate intake.

Alcoholic Neuropathy

RICHARD W. HORNABROOK, M.D.*

PERIPHERAL nerve disorders are a common sequel of excessive consumption of alcohol. The clinical features and natural history, the etiology and therapy of these disorders will be reviewed.

CLINICAL FEATURES

Alcoholic neuropathy can be defined as a generalized symmetrical affection of the peripheral nerves which commences in the extremities and spreads proximally. The disorder results in weakness and wasting of the muscles most evident at the periphery, with reduced or absent deep tendon reflexes. There is also an impairment of sensation so that there is a decreased ability to appreciate the vibrations of a tuning fork, the movements of the joints, the pain of a pinprick, and light touches on the skin. The sensory disorder is first detectable on the toes and fingers and later spreads up the limbs to produce a generally symmetrical and bilateral "glove and stocking" hypalgesia and hypesthesia.

The clinical characteristics, although presenting as a continuum, can be considered in three stages as this disease has a slow evolution. In the first instance, an aching discomfort and fatigue appear in the anterior tibial muscles on walking. As time passes, walking even shorter distances brings discomfort, painful cramps, and occasional paresthesias in the feet. These symptoms often persist for months. Examination of the patient at this time will reveal few abnormal signs: a little weakness of extension of the toes and ankles, some atrophy of the leg muscles with absent or reduced ankle jerks, and inability to appreciate the vibrations of a tuning fork at the ankle, and fine movements of the toes.

If untreated, the first symptoms intensify and extend from the feet to the legs, and the hands may become involved. The weakness of the ankles is so marked that foot drop renders the gait abnormal. Appreciable wasting of the leg muscles accompanies the weakness, and the knee and ankle reflexes are absent or sluggish. Weakness of the intrinsic hand muscles may be evident and the radial reflex may be sluggish. The patient complains of a persistent burning and coldness of the feet, and the finger tips feel tingling, rough, or numb. There is an inability to discriminate between two points on the finger tips along with some dullness of pain. Cutaneous sensibility is abnormal on the feet gradually merging with the normal below the knee. The disorder of position sense in the toes is marked, and a vibrating tuning fork is not felt at the shin.

Finally, in the severely affected patient, the legs are almost paralyzed and the hands are useless, the flabby muscles are tender to compression and fibrous contractures further limit mobility. All the deep tendon reflexes are absent. All forms of sensation are markedly impaired at the periphery so that a "glove and stocking" hypesthesia and hypalgesia or even anesthesia is produced. At the same time, a distortion of deep pain or pressure sensitivity may be present so that both light touch or pressure produces extreme discomfort. The peripheral fibres of the autonomic nervous system are also affected, and autonomic dysfunction is manifested by excessive perspiration of the instep and dorsum of the feet and on the finger tips; the skin is

From the Second (Cornell) Neurological Division, Bellevue Hospital, New York, New York.

* Present address: Neurologist, Wellington Hospital, Wellington, New Zealand.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

described as feeling cold and clammy. Less frequently the skin is dry, glossy and atrophic, the nails ridged, pitted and brittle. It was this advanced stage of the disease which featured in the classic descriptions of Lettsom[1] in 1787, and Jackson[2] in 1822.

Lettsom described the state of the patient vividly. "The appetite for food vanishes but sometimes continues voracious; and, at the same time whilst the body is costive, and no vomiting ensues, the lower extremities grow more and more emaciated; the legs become as smooth as polished ivory, and the soles of the feet even, glassy, and shining, and at the same time so tender, that the weight of the finger excites shrieks and moaning and yet, I have known, that in a moment's time, heavy pressure has given no uneasiness. The legs, and whole lower extremities, lose all power of action; wherever they are placed, there they remain till moved again by the attendant; the arms and hands acquire the same paralysis, and render the patients incapable of feeding themselves."

Just as the disease develops slowly so recovery is a gradual process. The most recent symptoms and signs are the first to clear whereas the earlier signs may change only very slowly. The loss of vibration sense of the ankles and the absent ankle jerks may persist as a permanent legacy of the illness.

However, the clinical findings in many cases of multiple neuritis do not conform to the conventional descriptions and the disorder is subject to great individual variation. Patients are encountered who have been troubled by an uncomfortable numbness of the feet for many months and in whom no evidence of weakness is found. In others, loss of the sense of the position of the joints with resulting ataxia may be the predominant disability. In still others, weakness and foot drop may be virtually the sole disability. Unilateral or asymmetrical neuropathies are sometimes encountered in chronic alcoholics. Dysfunction in the areas supplied by the musculospiral or peroneal nerve is most commonly observed. Cases have been recorded in which the supinator longus and the extensor metacarpal of the thumb were spared while the rest of the muscles innervated by the musculospiral nerve were severely paretic. Oppenheim[3] believed that the disorder was sometimes limited to individual nerves. He had observed patients with peroneal nerve palsies in whom tibialis anticus was spared. Sometimes the extensor communus digitorum was the only muscle affected, in such cases the paraesis were almost always symmetrical. While many of these asymmetrical peripheral disturbances may be attributable to pressure, a general metabolic etiology has not been excluded.

Rarely, cranial nerve palsies may be encountered in chronic alcoholism. These palsies are usually bilaterally symmetrical, but isolated palsies are sometimes met with. The seventh nerve is most frequently involved, the external ocular motor nerves less often. Pupils which are irregular and sometimes unreactive to light are found in alcoholics in the absence of any clinical or laboratory evidence for a syphilitic etiology. It should be borne in mind that in the absence of signs of generalized peripheral neuropathy, isolated cranial nerve palsies occurring in alcoholics may not be manifestations of a general metabolic defect.

Visual failure, with bilateral central scotomas, is occasionally encountered, and demyelinization of the optic nerves has been described. Most patients reveal some degree of mental change: their memory is poor, they have poor orientation, a decreased attention span, and lack of concentration; formal testing confirms an early dementia. The Korsakoff type of psychosis is much less frequently encountered than are mild defects of mentation. Along with these mental changes, the well recognized association of neuropathy with Wernicke's encephalopathy further confirms the fact that the central nervous system does not escape unscathed. The earlier writers do not allude to lesions of the spinal cord, but we have encountered several chronic alcoholics with peripheral neuropathy and unequivocal signs of corticospinal tract disease. In these patients a clinical diagnosis of subacute combined degeneration of the cord has failed to be substantiated by hematologic and radioactive cobalt studies.

Greenfield and Carmichael[4] found demyelinization of the peripheral nerves in alcoholic polyneuritis. Evidence of axis cylinder destruction was found at a variable time following the onset of the demyelinizing process. Zimmerman[5,6] made a study of the pathologic changes and found no evidence of an active inflammatory cellular reaction at any stage of the disease. Nonne[7] has described a loss of myelin in the dorsum and lateral columns of the spinal cords of chronic alcoholics, and he pointed out the close similarity superficially which these lesions had to those of combined system disease. Other writers[8–11] also report the occurrences of spinal cord lesions involving the long tracts of both the lateral and posterior columns. Similar changes have been encountered with peripheral neuropathy in the nutritional disorders of the severely malnourished.

The diagnosis of peripheral neuropathy does not usually present any difficulty, and the cause is usually so obtrusive that a diagnosis of alcoholic neuropathy may become inevitable. There is a danger of making a diagnostic error by attributing every neuropathy in an alcoholic to his way of life when other factors may well be responsible. Walshe[12] has emphasized the uniformity of the clinical picture of neuropathy despite the diversity of the causative factors and has said that there are no qualitative differences between the various types of peripheral neuropathy.

Different features have in the past been considered of value in the differentiation of alcoholic neuropathy from other neuropathies. Wilson[13] believed that the presence of increased sensitivity to deep pain and pressure was suggestive of alcoholic neuropathy, although he emphasized that it was not pathognomonic. Granger Stewart[14] was of the opinion that alcoholic neuritis could be recognized by the prominence of certain symptoms, namely, the severity of pain and cutaneous paresthesias, the tender muscles, the involvement of the lower limbs, the peripheral distribution of the symptoms and the prominence of involvement of the extensor muscles. Unfortunately, any or all of these symptoms may be present in neuropathies as diverse as those found in diabetes mellitus, polyarteritis nodosa, or infectious polyneuritis. Many writers have stressed the value of mental deterioration in the differentiation of alcoholic neuropathy from other neuropathies, and our experience supports this view.

Further, the nutritional and toxic neuropathies as a group rarely involve the proximal muscles of the limbs or the trunk musculature, and when both proximal and distal muscles are weak, the distal ones will always be found most severely affected. In general, however, it is wise to bear in mind that in individual cases there are no pathognomonic features which allow alcoholic neuropathy to be discriminated from neuropathy arising from other causes. In many cases stigmata of chronic alcoholism are encountered, the unkempt appearance and general tremulousness are striking. Nausea, vomiting, and abdominal discomfort suggesting gastritis or the signs of portal cirrhosis may be found. The disease is a general metabolic one, and substernal discomfort, tachycardia, and electrocardiographic abnormalities point to an accompanying cardiac abnormality.

ETIOLOGIC CONSIDERATIONS

Many authors[12,15,16] have emphasized that alcoholic neuropathy is a bilaterally symmetrical affection and imply that this symmetry involves the peripheral nerves in a generalized and nonselective fashion; this suggests a metabolic defect in its causation.

Neuropathy appears to be found in 3 to 20 per cent of patients admitted to the hospital with chronic alcoholism.[17] The figures from different institutions vary according to the criteria for selection of cases for admission. Formerly considered to be primarily a disorder of females, the great bulk of the cases today is found in males; this change in incidence is probably attributable to a change in social customs, rather than to any alteration in the incidence of the disease. Victor and Adams[17] have shown that the incidence of neuropathy in female alcoholics is higher than in males.

Gowers[18] and Oppenheim[3] were both struck with the importance of cold as a precipitating factor, and a higher incidence of neuropathy is found during the winter months. Inter-

current disease, bronchitis, and influenza are more common in colder weather, and it seems that the appearance of any infection in an alcoholic may be associated with the development of neurologic symptoms. Tuberculosis has this association to a marked degree, and a period of unaccustomed exertion or an attack of vomiting or of diarrhea may be followed by paresthesias or foot drop in a chronic alcoholic who was formerly symptom-free. It is easy to see why these agents were all held to be responsible for neuropathy in the past, whereas today they are known to increase the vitamin requirements and exaggerate a mild deficiency.

Physicians had always been perplexed by the question: "If alcohol or one of its metabolites was the toxic agent responsible for the generalized neuropathy, why then should neurologic damage develop in such a small percentage of chronic alcoholics?" With knowledge that beriberi was a deficiency disease, curable by the administration of vitamins, it was not long before Shattuck[19] postulated that alcoholic polyneuropathy might have a similar etiology. Wechsler,[20] after a careful comparison of the characteristics of beriberi and alcoholic neuropathy, came to the same conclusion. Minot, Strauss and Cobb[21] examined a number of alcoholic patients with respect to deficiency disease. They experienced great difficulty in assessing the diet of these patients because of the unreliable dietary histories. They came to the conclusion that most of their patients had had an inadequate diet for a considerable time.

In general, chronic alcoholics spend less money on food than nonalcoholics and the association of anorexia with excessive drinking is well known. The inadequacy of the diet may be enhanced by additional factors. Jolliffe et al.[22] have drawn attention to the intake of "vitamin free" calories in the form of alcohol. The disturbance in the ratio of vitamins to calories in the diet which results may render a barely adequate diet inadequate in vitamins.

Strauss[23] has discussed the importance of a gastrointestinal disorder in conditioning a deficiency disease. The great frequency of achlorhydria in chronic alcoholics may be of importance.[24] Blotner[25] has described the inhibition of proteolytic gastrointestinal enzymes by alcohol.

The part which cirrhotic liver disease may play remains uncertain, although Wayburn and Guerard[26] found a high association between neuropathy and cirrhosis.

The proof that dietetic factors are intimately concerned with the appearance of neuropathy was obtained by Strauss,[27] who maintained ten chronic alcoholics with peripheral neuropathy on their usual whiskey intake and supplemented their diet with vitamins administered parenterally and orally; in all patients the polyneuropathy improved. Blankenhorn and Spies[28] carried out a similar experiment with patients suffering from "alcoholic neuritis," and "alcoholic pellagra and neuritis." When given whiskey and a good diet they also improved.

For the sake of convenience the various dietary factors which may be implicated will be discussed in turn.

Thiamine has long occupied a central place in any discussion on the causation of alcoholic neuropathy. There is no doubt that thiamine is essential for the normal metabolism of nervous tissues. Peters[29] has shown that it is concerned in the release of energy from carbohydrate, and, as co-carboxylase, it is concerned with the oxidation of α-keto acids. In the absence of thiamine both pyruvate and pyruvic aldehyde accumulate, and with a high carbohydrate diet this accumulation is increased. Sinclair[30] has suggested that it is the accumulation of these toxic metabolites which produces the anorexia of thiamine deficiency. In thiamine deficiency the neurone may be disorganized either through its own inefficient metabolism or through the accumulation of toxic metabolites. However, attempts to produce thiamine deficiency in man,[31–33] have been hampered by the difficulty of restricting the deficiency to thiamine alone. Objective signs of neuropathy have seldom been observed and when substitution therapy was instituted the preparations given usually included several vitamins. In experimental animals changes in peripheral nerves do

develop in thiamine-deficient birds. In mammals it has been more difficult to produce experimental neuropathy, and it was not established until Sinclair and North[34] produced a peripheral nerve degeneration in rats on a thiamine-deficient diet. Thiamine is deficient in the diets of chronic alcoholics, and it is possible that in the presence of gastrointestinal disease its absorption may be impaired or that the vitamin may be inactivated. Further, in the presence of liver disease, the phosphorylation of thiamine in that organ may be inadequate.

The excretion of thiamine in alcoholics with neuropathy is subnormal.[35] Often, however, the alcoholic neuropathy does not improve strikingly with thiamine treatment alone, and Meiklejohn[36] seriously doubted whether this vitamin had any etiologic relationship. Walshe[37] has expressed similar views and Brown[38] found that patients with alcoholic neuropathy treated with thiamine left the hospital no sooner than patients treated with good diet alone.

Pantothenic acid is a part of coenzyme A and is also concerned with the oxidation of pyruvate.

Experimental pantothenic acid deficiency in swine has produced a peripheral neuropathy.[39] In man, Bean and Hodges[40] have given an analogue of pantothenic acid to volunteer subjects producing symptoms of peripheral nerve disease. This vitamin is extremely widely distributed in nature, and there is no evidence that it is deficient in chronic alcoholics.

Lipoic acid: The pyruvate oxidation system can be disordered in another way in chronic alcoholics if the production or catalytic function of lipoic acid is disrupted.

Sinclair[30] has stressed the importance of this substance in metabolism and has drawn attention to the fact that, as it is probably formed in the liver, production may be defective in a chronic alcoholic. A deficiency of lipoic acid may be produced by the formation of stable compounds with excessive pyruvate or the acetaldehyde formed during the breakdown of alcohol. Vitamin B_{12} may be concerned in the oxidation and reduction of lipoic acid, and a deficiency of vitamin B_{12} could occur in alcoholics.

Finally it is of interest that the neuropathy of chronic alcoholics treated with Antabuse® may be due to this chemical, blocking or combining with lipoic acid.

Pyridoxine: A deficiency of pyridoxine in the diet of swine has been shown to produce peripheral neuropathy,[39] and in man the antagonist desoxypyridoxine has produced peripheral neuritis.[41] The importance of this vitamin in the maintenance of a normal peripheral nervous system has been clearly shown by its use in the prevention and treatment of neuropathy occurring in patients receiving isoniazid therapy for tuberculosis.

Spies[42] found that in several patients with alcoholic neuropathy, who had not completely responded to treatment with nicotinic acid and thiamine, the neuropathy cleared up rapidly after pyridoxine was administered.

Riboflavin and nicotinic acid are known to be essential for the integrity of the nervous system. Alcoholics have been described with the clinical stigmata of riboflavin deficiency. The administration of the specific vitamin relieves the skin lesions, whereas the neuropathy is unaltered.[43] In nicotinic acid deficiency both the mental disturbance and the skin lesions clear rapidly following the administration of the specific vitamin, leaving the peripheral neuropathy unaffected.[44,45] On the basis of this evidence these vitamins have been thought to play no part in the causation of peripheral neuropathy. This argument, however, is not necessarily valid as there is good evidence that once morphologic changes in the peripheral nerves have occurred recovery may be very slow.

Vitamin B_{12} deficiency may produce peripheral neuropathy, and the presence of achlorhydria and gastrointestinal disorders in alcoholics render a defect in vitamin B_{12} absorption possible. There is no record of vitamin B_{12} deficiency in chronic alcoholics, although it seems quite possible that this may occur.

Vitamin C: Wortis et al.[46] have shown that chronic alcoholics with peripheral neuropathy may be deficient in this vitamin. They drew

attention to the fact that mental and neurologic signs are rare in scurvy and that scurvy seldom develops in alcoholics nor does alcoholic neuropathy respond to therapy with vitamin C.

Vitamin A: Mellanby[47] has found peripheral neuropathy in experimentally induced vitamin A deficiency. But there is no evidence that this vitamin is deficient in the chronic alcoholic patient.

Inanition: Several authors have reported peripheral neuropathy in animals given adequate amounts of vitamins but deficient in both calories and protein. The role played by the essential amino acids in the nutrition of the nervous system is quite unknown. It may well be that deficiency of these and other factors is also involved in the production of alcoholic neuropathy. The chronic alcoholic is almost always deficient in many different vitamins as well as other nutritional elements. The combination of these various factors may be more dangerous to the nervous system than the absence of any single substance, and these deficiencies may render individual nerves more susceptible to injury by other agents such as physical trauma.

TREATMENT

In the present state of knowledge it is impossible to be dogmatic about the treatment of alcoholic neuropathy. There is evidence that treatment with thiamine alone is not always satisfactory and Romano[48] found 6.4 per cent of patients with chronic alcoholic neuropathy did not respond to thiamine therapy; 32.4 per cent improved slightly, while the remainder improved markedly and were considered cured. Jolliffe[49] demonstrated that, in general, patients responded best to massive doses of thiamine and the speed of recovery was directly related to the amount of vitamin administrated. Because of the multiple deficiencies usually present in chronic alcoholics, there would seem to be good grounds for giving multivitamin preparations rather than single crystalline substances.

In the first week vitamins in ten times the normal daily requirement should be given, and thereafter about half this amount should be administered. There is very good evidence, both theoretic and clinical, for believing that a good mixed diet may be of equal value to the administered vitamins.

In the acute stage of the illness there is some evidence that brisk exercise may be harmful. Passive movements are important from the onset to prevent fibrous contractures and limitation of joint mobility. Voluntary activity should be postponed until the patient is convalescent.

On the regimen of parenteral vitamins, a good diet and bed rest almost all patients with alcoholic neuropathy recover, those with moderate severity in six to eight weeks, those with more serious involvement after a somewhat longer period.

CONCLUSION

In discussing this subject of alcoholic neuropathy I have attempted to draw the distinction between those facts which have been definitely established and those which are merely conjectural. The clinical characteristics of this disorder are now well known and clearly demarcated.

It is perhaps surprising that so much obscurity surrounds the precise etiologic agents. The time has arrived when a reappraisal of the nutritional factors and therapy of alcoholic neuropathy should be made.

REFERENCES

Because of the author's transfer to an overseas post, the references for this article were not received in time for publication. However, they will appear in the author's reprints.

Some Unusual Neurologic Diseases Complicating Chronic Alcoholism

ELLIOTT L. MANCALL, M.D.*

A NUMBER of neurologic disorders occur in people addicted to the excessive intake of alcohol in addition to the relatively common and well recognized Wernicke-Korsakoff syndrome and alcoholic polyneuropathy. It is not possible to state the incidence of these complications with any accuracy; in general, they tend to be much less frequent than either of the two just mentioned. Much confusion has existed concerning the identification and classification of these diseases, which have usually been grouped together under the vague term "alcoholic encephalopathies."

In recent years, however, it has been possible to isolate at least some of these disorders and to designate fairly precisely their clinical and pathologic features. Among these, cortical cerebellar degeneration, deficiency amblyopia, Marchiafava-Bignami's disease and central pontine myelinolysis are most readily defined; cerebral cortical atrophy may also be included, although this disorder is much less well delineated than the others. Although all of these differ from one another in many respects, both clinically and pathologically, there are certain features common to all.

First, these complications usually occur only after serious, steady drinking of at least several years' duration. Second, nutritional depletion is commonly present for months or even years before the onset of the illness. Third, although pure forms of these diseases are encountered, there is a decided tendency for them to occur in varying combinations with each other or with other disorders of recognized nutritional etiology, such as polyneuropathy, pellagra, and Wernicke's encephalopathy and Korsakoff's psychosis. Fourth, there is a preponderance of affection of males, seen most notably in cortical cerebellar degeneration. Fifth, with the sole exception of Marchiafava-Bignami's disease the clinical syndromes are remarkably stereotyped. Sixth, the pathologic alterations in each of these disorders are similarly stereotyped, demonstrating in a striking way two qualities which are consistently associated with diseases of known toxic or metabolic causation, namely, a constancy of localization and a bilaterally symmetrical distribution of the lesions.

The exact etiologic relationship of these entities to the chronic abuse of alcohol is still not clear. Clinical studies suggest that most are not caused by a direct toxic effect of alcohol upon the nervous system, but rather by nutritional depletion, the role of alcohol being a secondary one. The particular dietary deficiency responsible for any or all of these disorders has not yet been clearly defined, however, and attempts at producing them experimentally have as yet met with little success.

CORTICAL CEREBELLAR DEGENERATION

An evanescent ataxia of gait may be encountered in acute alcoholic intoxication and, rarely, in the withdrawal phase; and ataxia, usually short-lived, is a characteristic feature of Wernicke's encephalopathy. In addition to these relatively acute ataxic states, however, a chronic ataxic syndrome is not uncommonly encountered in the alcoholic patient. This syndrome, often and perhaps erroneously

From the Department of Neurology, Jefferson Medical College, Philadelphia, Pennsylvania.

* Assistant Professor of Neurology.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

referred to as "alcoholic cerebellar degeneration," has been recognized relatively frequently; although no reliable estimate of the absolute incidence of this disease is available, it represents in our experience the most common of the nonfamilial cerebellar degenerations. Despite the notorious difficulty in distinguishing between the various types of degeneration of the cerebellum and its connections on clinical grounds alone, this particular variety has a number of features which permit accurate diagnosis during life.[1]

In the majority of patients cerebellar symptoms begin before the age of fifty. In general, the patient has been drinking excessively and steadily for a number of years (usually ten or more) before the onset of cerebellar difficulties. The majority of the patients also present a history of faulty nutrition for months or years before symptoms begin, and on examination many of the accepted stigmata of undernutrition, such as loss of muscle bulk, redness and depapillation of the tongue are evident. Signs of recognized nutritional disorders, particularly polyneuropathy and Wernicke's encephalopathy, are often found. The initial complaint is almost invariably one of a disturbance of gait or of balance, other neurologic symptoms being absent or minimal throughout the course of the illness. Similarly, the most prominent abnormality demonstrable on examination is a disorder of gait and stance. An ataxia of gait and of individual movements of the legs is invariably present; truncal instability is very common. Rapid postural adjustments are particularly difficult. Few of these patients are so incapacitated as to be confined to bed, but most require some support for standing or walking. In contrast to the severe disturbance of the lower extremities, the arms are in general only mildly affected. A mild slowing or slurring of speech may be present, but severe dysarthria is rare. Nystagmus, even of a mild degree, is uncommon; occasionally, ocular dysmetria may be observed. Aside from signs referable to a coexisting neurologic disease, such as mild polyneuropathy, Wernicke's disease or Korsakoff's psychosis, no other abnormalities are seen. A slight elevation of spinal fluid protein may be present.

One of the most characteristic clinical features of this illness concerns the mode of progression of the cerebellar syndrome. In at least half the patients so afflicted, the disease evolves fairly rapidly, the maximum neurologic deficit being attained in a matter of days, weeks or months. Once this maximum intensity has been reached, a period of clinical stability ensues, the cerebellar symptom complex remaining virtually unchanged for many years thereafter. In a smaller proportion of the cases, the disease may progress for several years or longer. but ultimately most of these patients also reach a clinical plateau with no further notable progression. Occasionally, one encounters a patient who has had a mild and stationary deficit for many years, who then experiences a rapid worsening in his condition, followed once again by stabilization. In a general sort of way, progression of the illness appears associated with continued drinking and faulty nutrition, whereas stabilization seems associated with abstinence and improved dietary intake. This is not invariably true, however: this illness has developed in some patients while abstinent (as during hospitalization for delirium tremens, febrile infectious disease or, as in one case, in the course of prolonged institutionalization for Korsakoff's psychosis), and has apparently stabilized in a few patients who continued to drink as much, and eat as poorly, as before.

Just as the clinical features of this illness are remarkably stereotyped, so too the pathologic changes are strikingly similar from case to case. The essential change is a degeneration, in varying degrees, of all neuronal elements of cerebellar cortex (Figs. 1 and 2). When the disease process is most severe, the Purkinje cells, intrinsic nerve cells of the molecular layer, granule cells and the intrinsic nerve fiber networks, are indiscriminately destroyed, and there is marked shrinkage of the affected folia; lesser degrees of severity can be discerned, the process tending to shade off gradually into normal parenchyma. The Purkinje cells seem to be the most sensitive cellular elements, since, in less severely affected regions,

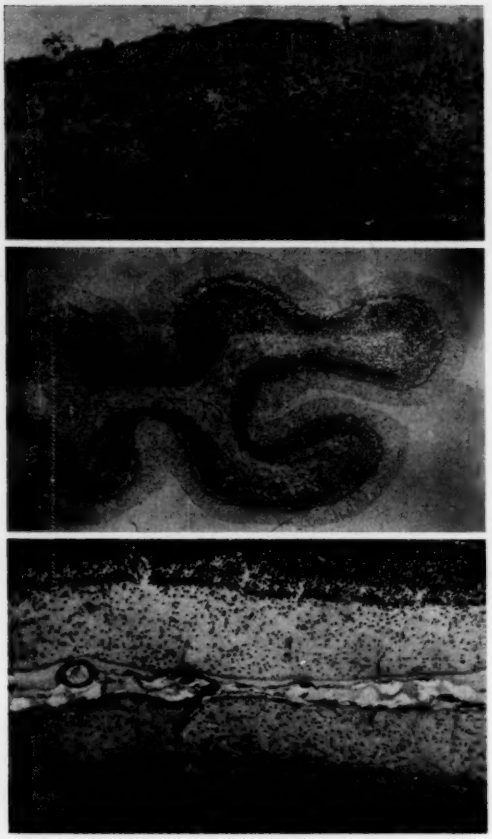


FIG. 1. Cortical cerebellar degeneration. Varying degrees of neuronal loss in superior vermis. *Top*, loss of all intrinsic nerve cells of molecular layer with marked shrinkage of this layer, complete loss of Purkinje cells, and patchy but severe loss of granule cells (oil red O stain). *Middle*, severe loss of Purkinje cells, some shrinkage of molecular layer, and a moderate loss of granule cells, particularly at the crests of the involved folia (cresyl-violet stain). *Bottom*, marked loss of Purkinje cells, with intact molecular and granular layers (cresyl-violet stain).

a loss of these cells is apparent before any significant changes in the other neurocellular elements of the cerebellar cortex can be discerned. Glial changes reflect the tempo of the illness, fibrous gliosis being common and phagocytic activity inconspicuous. Inflammatory changes have not been encountered.

Particularly noteworthy is the remarkable restriction of the changes to the anterior and superior aspects of the cerebellar vermis and

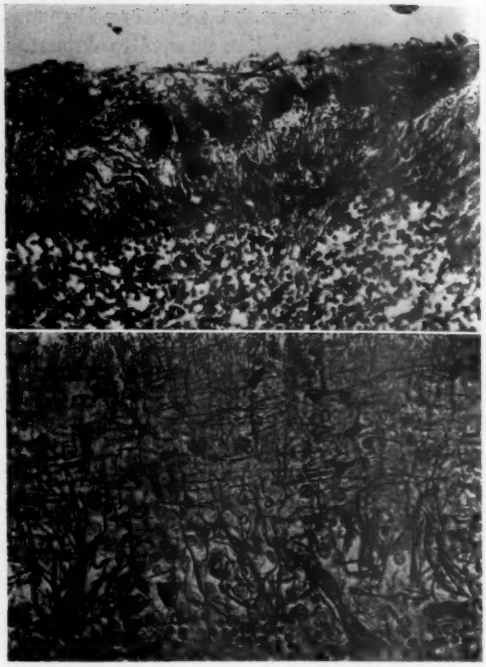


FIG. 2. Cortical cerebellar degeneration. *Top*, loss of all intrinsic fiber systems of the cerebellar cortex, with formation of glial-adventitial whorls in a very severely affected portion of superior vermis (Cajal reduced silver method). *Bottom*, preservation of these fiber systems in an area less severely involved. Note the empty peri-Perkinje basket formations (Cajal reduced silver method).

hemispheres (Fig. 3). The superior vermis, particularly lingula, central lobule and parts of the culmen, are almost invariably affected, and that portion of the declive bordering the primary fissure is often involved as well. In contrast, the remainder of the vermis is little affected, if at all. Similarly, the changes in the cerebellar hemispheres, seen in a number of these cases, exhibit a striking topographic restriction to the more anterior portions of the anterior lobes and less often, the simple lobules.

Secondary changes may be observed in the lamellar white matter and in the deep cerebellar nuclei, particularly the fastigial, globose and emboliform, in keeping with the pattern of corticonuclear projections from the involved folia. The olivary complex is almost always involved, the vestibular nuclei only occa-

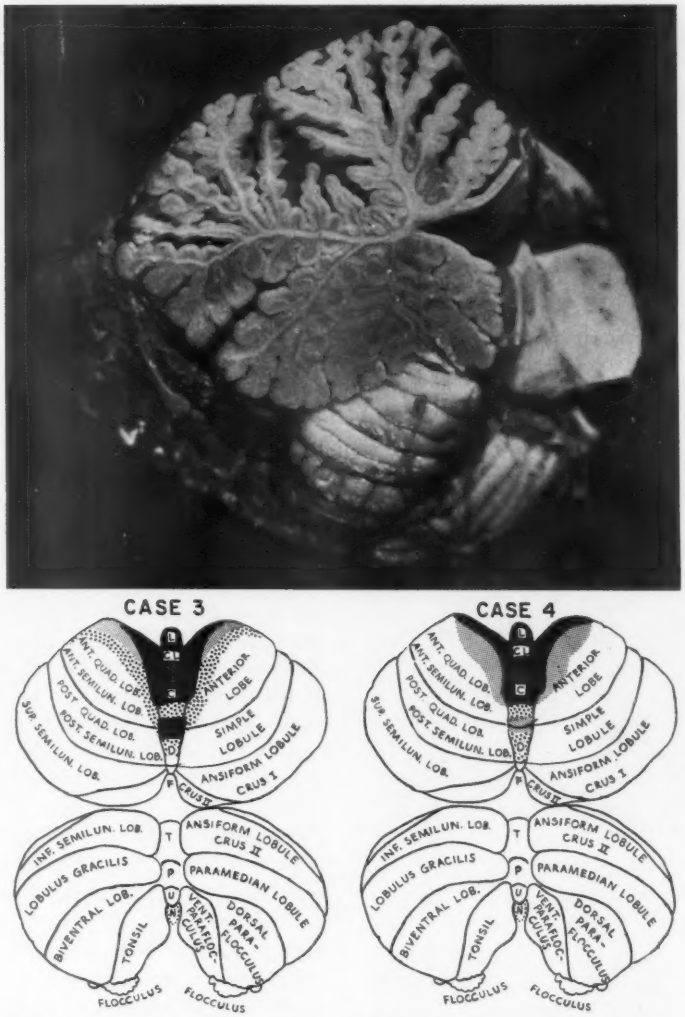
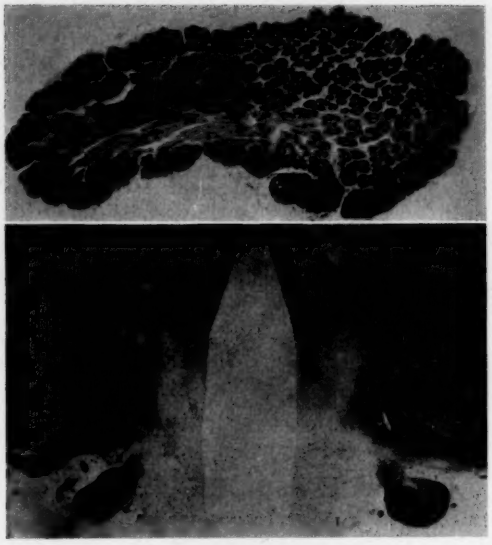
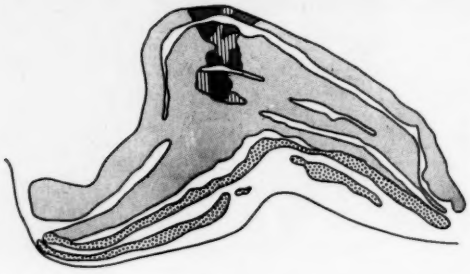


FIG. 3. Cortical cerebellar degeneration, illustrating the selective involvement of the anterior and superior portions of the cerebellum. *Top*, sagittal section through vermis showing shrinkage of folia and widening of sulci in the superior vermis. *Bottom*, diagrammatic representation of the involvement of the cerebellar cortex from two typical patients with this disorder. The severity of affection is indicated by the density of the shading.

sionally. Other nuclear groups of the brain stem are not affected.

It is recognized that abuse of alcohol is common to all of these patients, a fact which has led others to the supposition that this disease is caused by direct toxicity of the alcohol itself. However, since some patients have clearly been abstinent for some time prior to the development of cerebellar symptoms, it is difficult to accept alcohol itself as the direct causative agent. On the other hand, the great majority of these people also suffer from chronically poor nutrition, and, in fact, a profound weight loss may immediately precede the onset of symptoms, emphasizing the probable significance of nutritional depletion





SEVERE MODERATE

FIG. 4. Deficiency amblyopia. *Top*, optic nerve, demonstrating loss of myelinated fibers in the region of papillomacular bundle (Spielmeyer myelin stain). *Middle*, symmetrical degeneration of the papillomacular bundle is evident in the optic tracts (Loyez myelin stain). *Bottom*, diagrammatic representation of loss of nerve cells in the lateral geniculate bodies, in the portion of these nuclei within which the papillomacular bundles terminate.

in the production of this disease. A scattering of clinical reports of cerebellar syndromes occurring in persons with a background of malnutrition without alcoholism further support the contention that undernutrition *per se* may be the significant etiologic factor[2-5]; unfortunately, no pathologic studies of cases of this type are available. Thus, clinical experience at least suggests that nutritional deficiency of some sort is responsible for this cerebellar syndrome, and that the role of alcohol itself is largely secondary. Attempts to produce nutritionally-induced cerebellar disease in the experimental animal have shed little light on the problem of etiology; the few changes which have been reported are either very subtle[6,7] or devastating and acute,[8] and of uncertain significance.

DEFICIENCY AMBLYOPIA

For many years, a characteristic disorder of vision has been recognized in people who drink to excess. The clinical pattern of this disorder, commonly referred to as tobacco-alcohol amblyopia, is quite stereotyped.[9] Almost all patients so afflicted complain of a blurring or dimness of vision, and of difficulty in reading small print; an occasional patient may complain of difficulty in differentiating red from green. The disease usually evolves over a period of several weeks to several months. Examination confirms the presence of a loss of visual acuity, which may be quite profound, and a scotoma is almost invariably present on testing of the visual fields. The scotoma, usually bilateral and roughly symmetrical, may be central, centrocecal or paracentral. In general, the defect for red or green test objects is larger than that for white objects of equal size. Peripheral fields are intact. Funduscopic examination may be normal, or may demonstrate a mild to moderate degree of pallor of the optic nerve heads, most apparent in the temporal half of the discs. Aside from signs referable to other coexisting diseases, such as polyneuropathy or the Wernicke-Korsakoff syndrome, no other neurologic abnormalities are found. The cerebrospinal fluid protein may be slightly increased.

The great majority of patients suffering from this disease have been confirmed alcoholics for many years before the onset of the visual loss, and, in addition, most of them have also used tobacco in excess. An inadequate dietary intake is the rule, and a very severe loss of weight may just precede the development of the ocular symptomatology. Objective stigmata of undernutrition are commonly en-

countered. Improvement in the patient's visual acuity and reduction in size of the scotomas almost always follow upon the institution of an adequate dietary and vitamin intake.

Pathologic studies of this condition have been infrequent. The basic change is a loss of myelin sheaths and axis cylinders, with replacement gliosis, in the optic nerves, chiasm and optic tracts corresponding in location to the course of the papillomacular bundles (Fig. 4). A loss of neurones with secondary gliosis may be seen in the portions of the lateral geniculate bodies within which the papillomacular bundles end; this presumably is due to transynaptic degeneration. Changes in the optic radiations and striate cortex have not been seen. The status of the retina itself is a matter of some dispute: in some reported cases of this condition, no abnormalities of the ganglion cells have been found, [10–12] whereas in others a loss of ganglion cells has been noted in the macular regions.[13–15] Similarly, the question as to whether the disease begins in the retina or in the optic nerves is as yet unsettled; however, the finding of an alteration in the optic nerves with intact retinas in some cases would suggest that the primary affection is one of the optic nerves themselves.

Despite the element of toxicity implied in the term tobacco-alcohol amblyopia, the bulk of the evidence favors the interpretation that this disease is nutritional in origin. Most, if not all, of the patients have, as stated, a poor dietary history, and often exhibit frank stigmata of nutritional depletion. Diseases of recognized nutritional origin, such as alcoholic polyneuropathy and Wernicke's encephalopathy[9] or pellagra[16–20] are not infrequently found in conjunction with the amblyopia. Carefully documented clinical studies, such as those of Carroll,[21,22] indicate that recovery from the amblyopia may occur despite persistence of the premorbid drinking and smoking habits, provided the patients receive an adequate diet and/or B vitamins. Finally, under conditions of severe nutritional deprivation without concomitant abuse of alcohol, as in prisoner of war camps, a deficiency amplyopia occurs which is clinically indistinguishable from the

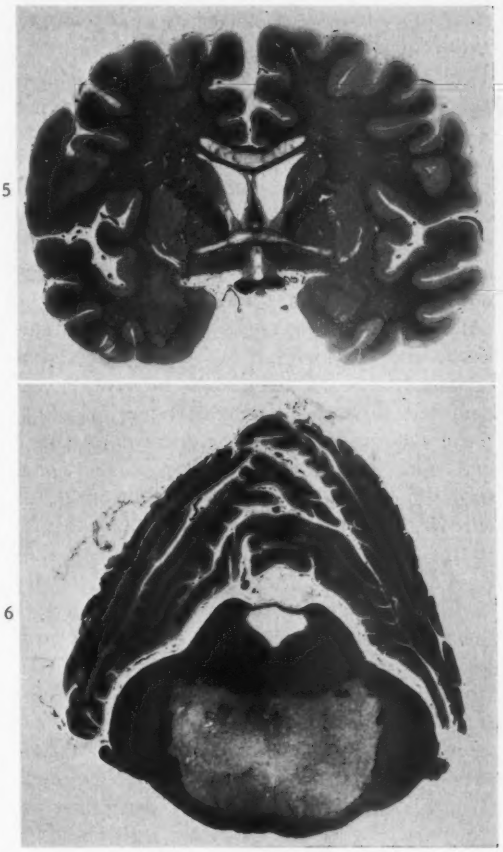


FIG. 5. Marchiafava-Bignami's disease. Note the symmetrical degeneration in both the corpus callosum and anterior commisure (Loyez myelin stain).

FIG. 6. Central pontine myelinolysis. An extensive symmetrical region of demyelination is evident in the basis pontis (Loyez myelin stain).

amblyopia observed in alcoholic patients.[23–28] In the few pathologically verified instances of this sort,[29] the morbid changes are similar to, if not identical with, the changes which have been recorded in alcoholic patients.

It would thus appear firmly established that tobacco-alcohol amblyopia has a nutritional origin. The specific dietary lack, however, is as yet uncertain. Thiamin[21,30] riboflavin[22] and vitamin B_{12}[31] have all been implicated by various authors, but no single report is conclusive. It is possible that, under given circumstances, a deficiency of any one of these

substances may produce the same effect upon the optic nerves.

MARCHIAFAVA-BIGNAMI'S DISEASE

Marchiafava-Bignami's disease, or primary degeneration of the corpus callosum, is a rare complication of chronic alcoholism. It was originally considered to occur primarily in middle aged or elderly males of Italian descent addicted to crude red wine; however, none of these conditions are necessary for the occurrence of this disease. The clinical manifestations are diverse and nonspecific, and include emotional disturbance of many sorts, dementia, aphasia, seizures, tremor, rigidity, paralysis and, terminally, deepening stupor and coma. The course may be a subacute one, evolving over a period of several months, or chronic, lasting several years. In contrast to the diversified clinical syndrome, the pathologic changes are quite stereotyped and consist basically of a symmetric degeneration of myelin sheaths in the mid-zone of the corpus callosum, beginning in the most anterior part of this structure and extending caudally (Fig. 5). Axic cylinders are often relatively well preserved, although occasionally frank cavitation with total tissue destruction has been noted. Gliosis is common, the severity depending on the chronicity of the process. Inflammatory changes are minimal or absent. Similar change may be seen in the central portion of the anterior commissure, in the central and convolutional cerebral white matter, in the optic chiasm and radiations, and in the middle cerebellar peduncles. It is probably unjustified to assert that this is a disease of commissural fiber systems, as has often been stressed, in that the changes are not strictly confined to such systems.

The etiology of this condition is not known. Constitutional factors, related to the frequent occurrence of the disease in Italian males, have often been suggested as playing an important role, at least in terms of predisposition; however, insofar as cases have occurred in Cuban,[32] Swiss,[33] French[34–37] and English[38] people with no known Italian ancestry, this is of dubious significance. Since the cases usually occur in people with a background of chronic alcoholism, a direct toxic effect of alcohol itself has generally been accepted as the cause. The experimental studies of Testa,[39] in which lesions similar to those described were produced in dogs by the chronic ingestion of alcohol, are often cited in support of this thesis; these findings, however, are open to serious question for a variety of technical reasons, and they have never been substantiated by other workers. On the other hand, most reports of this condition allude to a serious state of nutritional depletion. Furthermore, cases have been reported in which the characteristic changes of Marchiafava-Bignami's disease have been found in conjunction with disorders of recognized nutritional etiology, particularly Wernicke's encephalopathy[32,24–37] and perhaps pellagra as well.[36] Finally, there are several pathologically verified instances of this entity in which there was no history of alcoholism.[40,41] The available clinical evidence suggests therefore that this condition is probably not caused by a direct toxic effect of alcohol, but rather by nutritional depletion. No more definitive statement can be made at present; Bohrod's supposition that this is essentially a result of athiaminosis[42] has not been confirmed.

CENTRAL PONTINE MYELINOLYSIS

This term refers to an extremely rare complication of chronic alcoholism characterized by demyelination restricted to the basal portion of the mid and upper pons, symmetrically disposed about the mid-line[43] (Fig. 6). All myelinated fibers within the lesion, regardless of site of origin or termination, or function, are destroyed; in contrast, the intrinsic nerve cells and the axis cylinders of the basis pontis are almost entirely preserved, although qualitative changes in both elements may be seen. Phagocytic and astrocytic reactions appear appropriate to the presumed age of the lesion; no vascular or inflammatory changes are seen. The disease process appears to begin in the midline and to grow by a marginal advance in a symmetric fashion. When the lesion is large enough to produce clinical signs, the pattern is relatively stereotyped: flaccid quadriplegia, weakness of the tongue, inability to speak or swallow. Emotional lability and

forced crying have been observed, as well as loss of corneal reflexes. The course is progressive and rather acute, the patients dying within two to three weeks of the onset of the illness.

The disease generally occurs in people with a background of chronic, severe alcoholism, and malnutrition has been evident universally. Clinical and/or pathologic evidence of polyneuropathy and Wernicke's encephalopathy have been noted as coexisting with the pontine disease. On the basis of this data alone, the relative etiologic role of alcohol itself or of nutritional depletion cannot be determined. However, one such patient has been described[43] with no history of alcoholism, profound malnutrition developing on the basis of scleroderma of the gastrointestinal tract; the lesion in this instance was remarkably similar to those described herein. Two other cases have recently been recorded,[45] one in a child, the other in a nonalcoholic young female; nutritional depletion was suggested in both of these instances as well. Such cases suggest that malnutrition is the pertinent etiologic factor, although no specific dietary deficiency has been identified.

CEREBRAL CORTICAL ATROPHY

It is a commonplace observation that the brains of young and middleaged alcoholics often exhibit a degree of convolutional atrophy beyond that which would be expected for the chronologic age. This is usually of only mild to moderate severity, and tends to predominate in the more anterior portions of the cerebrum, although on occasion a generalized variety may be encountered. The atrophic changes are usually accompanied by some thickening of the overlying meninges and a mild symmetric compensatory dilatation of the ventricular system. Microscopically, no specific abnormalities are recognizable, although one often has the impression of a patchy but slight neuronal loss and glial hyperplasia. Qualitative changes in the cortical nerve cells, such as pigmentary degeneration, are common, but of uncertain significance. Morel[44] and others[33] have described a more "specific" type of cortical change, termed laminar cortical sclerosis, said to be characterized by nerve cell loss with astrocytic proliferation largely confined to the third cortical stratum, and particularly marked in the frontal regions. The clinical correlates of these various atrophic changes are as yet unclear; it is possible, but as yet unproved, that some degree of intellectual deterioration may be due to these morphologic alterations. The entire problem is in need of careful and detailed clinical and pathologic study. The factor, or factors, which are of significance in the etiology of these changes are completely unknown.

ACKNOWLEDGMENT

Figures 1, 2 and 3 are reprinted from Victor, M., Adams, R. D. and Mancall, E. L. *Arch. Neurol.*, 1: 579, 1959[1]; Figure 4 from Victor, M., Mancall, E. L. and Dreyfus, P. M. *Arch Opth.*[9]; Figure 5 from the collection of the Warren Museum and the Department of Neuropathology, Harvard Medical School; and Figure 6 from Adams, R. D., Victor, M. and Mancall, E. L. *Arch. Neurol. & Psychiat.*, 81: 154, 1959.[43]

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DISCUSSION

DR. JOSEPH M. FOLEY (*Jersey City, New Jersey*): Because both Dr. Mancall's paper and Dr. Hornabrook's paper have so much material about which there will be room for questioning and discussion, I would like to lead off with one question.

Dr. Mancall, do you think there is any possibility that the early stages of your central pontine myelinolysis might be related to the syndrome that was described some years ago by Dr. Norman Jolliffe under the title of nicotinic acid deficiency encephalopathy? If not, then where would you place such a syndrome, if you believe it to exist? There are people here who believe it to exist whether or not it can be identified as strictly nicotinic acid deficiency.

Now, are there any other questions or points of disagreement that people would like to make?

DR. MAURICE VICTOR (*Boston, Mass.*): Just two small points: At the suggestion of Dr. Folkers and Dr. Ritter of the Merck Institute, we tested the effect of lipoic acid in patients with the acute symptoms of Wernicke's disease and in animals with thiamine deficiency. In neither instance were we convinced that it exerted a therapeutic effect. In regard to the occurrences of pyramidal tract signs several factors have to be considered. Many alcoholics fall on their necks, injure their spinal cord and so, either immediately or later, show the effects of injury to the spinal cord. I think this may account for the extensor responses in some instances.

A small number of patients with delirium tremens or or Wernicke's disease may show extreme rigidity of the muscles and extensor plantar responses. These may be examples of the so-called nicotinic acid deficiency encephalopathy but we have not been able to prove a direct relationship to nicotinic acid deficiency.

On the basis of the very limited pathologic data that are available it would appear that the occasional example of spinal cord degeneration in alcoholics is truly a system disease. That is to say, the posterior column affection represents a retrograde degeneration secondary to peripheral nerve affection; the pyramidal tract lesion may be secondary to disease of the motor cells of the cerebral cortex, as has been described in pellagra. This type must be clearly distinguished from subacute combined degeneration of the cord which accompanies pernicious anemia. This is due to vitamin B_{12} deficiency and from the process just considered. Vitamin B_{12} deficiency causes a patchy diffuse affection of the white matter of the cord, which is intrinsic to the cord and not secondary to disease elsewhere in the nervous system.

DR. FOLEY: Dr. Hornabrook, would you like to make any extension of your remarks? Dr. Mancall, would you like to answer some of the questions that have been raised?

DR. ELLIOTT L. MANCALL (*Philadelphia, Pennsylvania*): I am not sure what nicotinic acid encephalopathy is. I have never personally seen a case recognizable as such, but that is of no great significance, I am sure. I did not mean to imply that these four or five diseases exhausted the repertoire, if you will, of "alcoholic encephalopathies." I suspect there are many more diseases about which we know little or nothing at the present time. However, I think we should be very reluctant to accept diseases as diseases on the basis of clinical description alone; of necessity we have to use pathology as our guide. Descriptive neuropathology must remain an indispensable tool in this respect. It is, of course, possible that there are reversible diseases which will never show themselves pathologically, and perhaps nicotinic acid encephalopathy is one of them. We should, however, be very careful in our acceptance and interpretation of such diseases. We are just beginning to work out the pathology of some of these disorders in a systematic way. Until such a time as more exhaustive and detailed studies are completed, we shall have to keep an open mind about many of these things.

DR. FOLEY: The more clinical part of our discussion has been presented. We plan to now go on to an item of less practical clinical importance at the present time, and to point out an area of investigation that over the next several years may throw great light on the practical clinical problems that we have been discussing to date.

Effects of Thiamine Deficiency on the Central Nervous System

PIERRE M. DREYFUS, M.D.* AND MAURICE VICTOR, M.D.†

IN the United States, nutritional diseases of the nervous system are virtually confined to the alcoholic population. Although only a small proportion of alcoholics is thus afflicted, the nutritional disorders assume significant proportions simply because of the prevalence of alcoholism.[1,2] They are made further important by virtue of their serious damaging effects on the nervous system, particularly upon "the mind." The role of thiamine in these nutritional disorders is unique, in that a deficiency of this vitamin alone has been shown to account for the ophthalmoplegia, ataxia and nystagmus of Wernicke's disease, and possibly for the amnestic symptoms of Korsakoff's psychosis, and polyneuropathy as well.[3,4] In this report our remarks will be concerned mainly with the effects of thiamine deficiency on the *central* nervous system. The effect of thiamine deprivation on the peripheral nervous system will not be considered in any detail since this represents a large and controversial subject in itself.

The human athiaminotic state, as exemplified by the Wernicke-Korsakoff syndrome, presents a number of interesting and as yet unsolved problems. The pathologic process has a selective predilection for bilaterally symmetrical parts of the nervous system, namely, the mammillary bodies, the medial portions of the thalamus and hypothalamus, the periaqueductal region of the mid-brain, and certain structures in the floor of the fourth ventricle. The lesions produced in animals deprived of thiamine bear a distinct resemblance to the lesions of Wernicke's disease, in regard to their topography and bilateral symmetry as well as their histologic character. The manner in which thiamine deficiency produces its effects on the nervous system and why certain anatomic sites are particularly vulnerable to these effects are problems awaiting solution.

In an attempt to get at some of these fundamental problems, we have undertaken a series of pathologic and biochemical investigations on experimental thiamine deficiency and its effects on the nervous system. In addition to reporting the results of these initial studies, we propose to review briefly the subject of experimentally induced thiamine deficiency as it pertains to the central nervous system.

THE CLINICAL AND PATHOLOGIC FEATURES OF THIAMINE DEFICIENCY IN ANIMALS

This has been the subject of a large number of reports since the original studies of Eijkman in 1897.[5] Most of the studies performed before the B vitamins became available in purified form are based on an erroneous nutritional premise. The deficiency was generally produced by the addition of autoclaved yeast to a diet lacking all B vitamins and it was assumed that the autoclaving process selectively destroyed only the thermolabile thiamine. Recently it has been established that while most of the thiamine (92 per cent) is indeed lost by the autoclaving procedure, other

From the Department of Neurology and Psychiatry (Neuropathology), Harvard Medical School, the McLean Hospital Research Laboratory, Waverley, Massachusetts, and the Neurology Service, Massachusetts General Hospital, Boston, Massachusetts.

* Instructor in Neurology; † Assistant Clinical Professor of Neurology.

This study was supported in part by grant B-1906 from the National Institute of Neurological Diseases and Blindness.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

vitamins are destroyed in addition, particularly folic acid (62 per cent) and riboflavin (54 per cent).[6] It follows that the only acceptable studies on induced thiamine deficiency are those which utilize synthetic diets to which all essential minerals and vitamins, except thiamine, have been added. In our own studies on rats, we adhered to this nutritional principle.

RATS

When *rats* are given a thiamine-deficient diet,* they gain weight and develop normally for two to three weeks, at the end of which time a decrease in spontaneous activity and a reduction in appetite sets in. After a period of steady weight loss lasting another two to three weeks, the animals show a number of abnormalities of posture and equilibrium: The back is arched, the hind legs are extended and stiff; movements are greatly restricted and take the form of a circling, the forepaws being used as a pivoting point. Frequently, there is a rhythmic instability of the head and trunk. The most advanced abnormality consists of a complete inability to make appropriate postural adjustments. A gentle twirling of the animal by his tail, for example, results in a series of uncontrolled rolling movements; after the resumption of the upright position, all four limbs are splayed far apart and exhibit rapid, irregular, quivering motions. Frequently, retraction or sharp forward flexion of the head is noted. At this stage of depletion, death may ensue within twenty-four hours unless the animal receives specific treatment.

These neurologic signs can be promptly reversed by the administration of 5 to 10 μg. of thiamine, although the animal remains somewhat slow in its movements and far below its optimal weight. If no more thiamine is given, severe symptoms reappear in three to six days. When suboptimal amounts of thiamine are added to the diet (up to 0.5 mg. per kg. of diet) a similar but somewhat slower-evolving clinical picture is observed.

Pathologic examination of the nervous systems of the rats, which had experienced several bouts of deficiency before being sacrificed, disclosed a number of abnormalities. About one-third of the animals showed a discrete, symmetrical area of pannecrosis in the dorsal pontine tegmentum, in the region of the lateral vestibular nucleus of Deiters (Fig. 1). The lesion consisted of a central core of tissue destruction and a marked proliferation of glia (Fig. 2). Many of the neurones showed varying stages of destruction. Within the center of the lesion all medullated fibers were destroyed (Fig. 1B). A prominent astrocytic hyperplasia was also observed in many other brain stem nuclei. Occasionally macrophages containing blood pigment were seen in the proximity of small blood vessels located in nuclei of the pontine tegmentum. The remainder of the brain and spinal cord appeared to be normal.

Pathologic changes in the central nervous systems of vitamin B_1-deficient rats were originally described by Prickett[7] and later by Kalm et al.[8] The former author described bilaterally symmetrical foci of hemorrhage in the floor of the fourth ventricle, which appeared to be acute and were accompanied by neither phagocytosis nor other evidence of reaction. The nuclei most affected by the hemorrhages were the solitarius and the vestibular group. In addition, the animals which had been subjected to recurrent bouts of deficiency were said to show qualitative alterations of the nerve cells and occasional foci of neuroglia near the chief vestibular nucleus.

It would appear that the lesions described

* The thiamine-deficient diet employed in our experiments had the following composition:

	10 kg. contained:
Vitamin free casein	2,000 gm.
Sucrose	7,100 gm.
Vegetable oil	400 gm.
Cod liver oil	100 gm.
Salt mixture, Hegsted IV	400 gm.
Choline chloride	4 gm.
Riboflavin	112 mg.
Calcium pantothenate	280 mg.
Niacin	560 mg.
Pyridoxine hydrochloride	56 mg.
Folic acid	14 mg.
Menadione	14 mg.
Vitamin B_{12}	180 mg.

Thiamine content was adjusted to contain between 0.1 mg. per kg. and 0.5 mg. per kg. (1 mg. of thiamine per kg. of diet is required for normal growth and development).

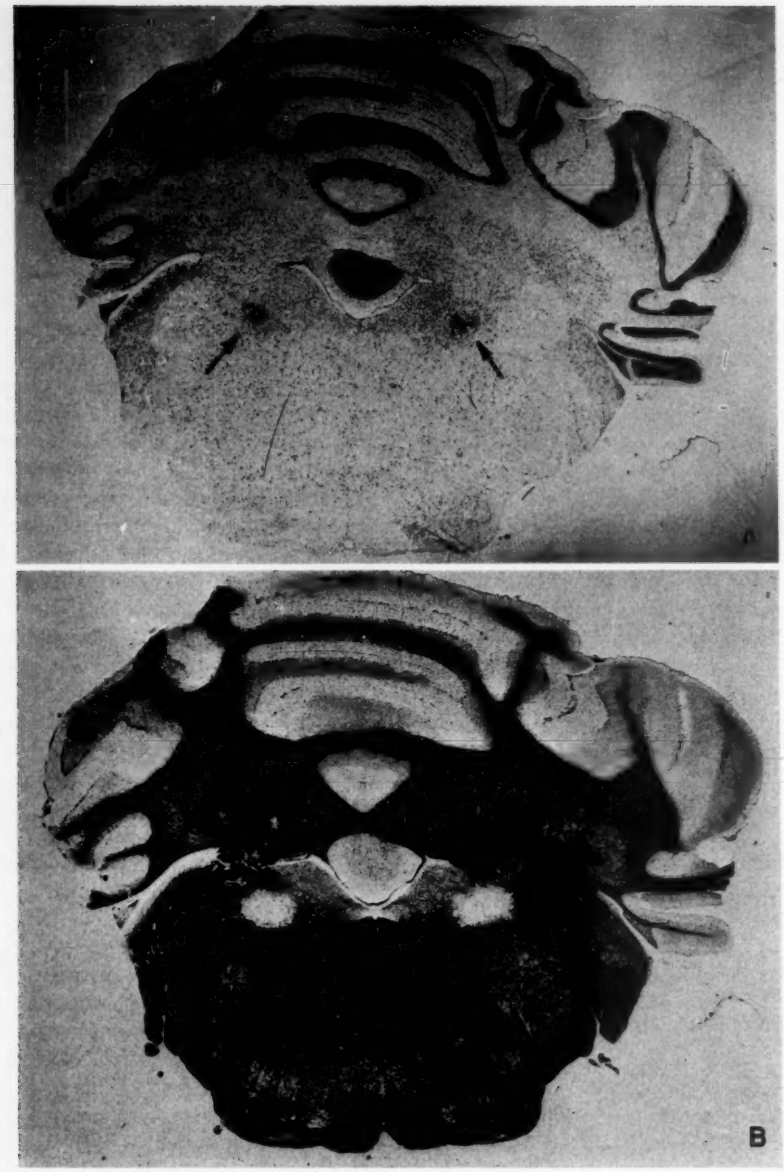

FIG. 1. The effect of thiamine deficiency on the brain of the rat. A, coronal section through the pons showing a symmetrical area of pannecrosis in the region of the lateral vestibular nucleus of Deiters. Nissl stain. B, the same section as in (A) stained for myelin. Loyez stain.

by Prickett differ substantially from our own. However, one of his illustrations discloses a cellular reaction in the region of the vestibular nucleus quite similar to the one observed by us. The apparent difference, therefore, may not be fundamental but may merely represent a difference in emphasis. The observations of Kalm et al.[8] more closely resemble our own.

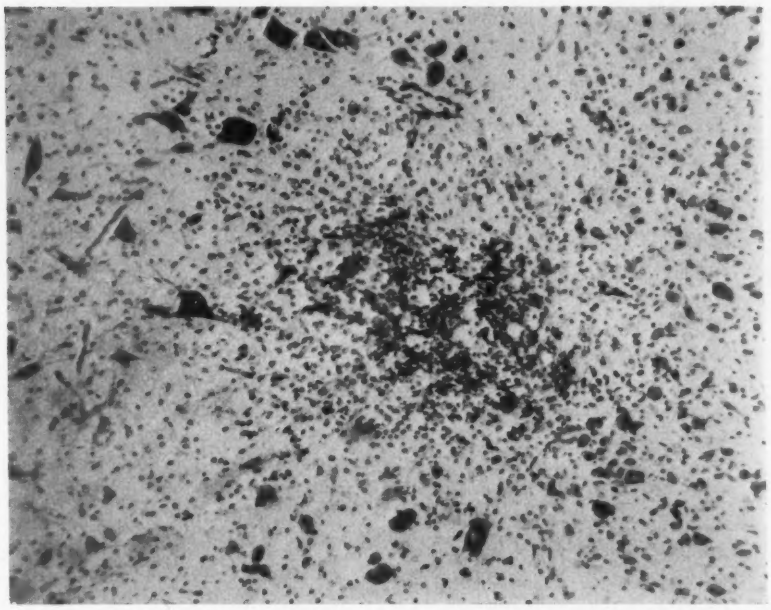

FIG. 2. Microscopic details of a typical lesion in the lateral vestibular nucleus, characterized by gliosis, proliferation of adventitial histiocytes and macrophages. Nerve cells are relatively preserved.

The brains of rats in which several bouts of deficiency had been allowed to develop showed "symmetrical, perivascular, mesodermal and glial cell aggregates associated with a loss of neurones involving the vestibular region." These authors also described a diffuse vacuolar degeneration of neurones, mainly within nuclei of the cerebellum, medulla and floor of the fourth ventricle and, to a lesser extent, neurones of the cerebral cortex and basal ganglia. It was difficult to distinguish the latter alterations from nonspecific agonal changes and postmortem artefact, particularly since many of the animals had not been allowed to die naturally.

PIGEONS

Lesions of the central nervous system due to thiamine deficiency can be readily produced in *pigeons*, the most important studies in this regard being those of Alexander and his colleagues[9,10] and of Swank and Prados.[11] Alexander and his associate have pointed out that lesions of the Wernicke type rarely occur if pigeons are kept on an entirely vitamin-free diet, but they can be produced with significant regularity if the diet is deficient in vitamin B_1, and other vitamins (A, B_2, C and D) are fed in excess. The lesions consisted of "pin-point hemorrhages within areas of subacute necrosis," and "affected the paramedian and paraventricular nuclei of the thalamus and hypothalamus and the region of the eye muscles." The nerve cells within these lesions were said to be damaged, and there was an increase in glial elements of all types. Alexander stressed the irregular dilation of the blood vessels within these lesions and the increase in their endothelial and adventitial elements. He concluded that "angiodegeneration with varicose deformity of the vascular bed is the primary change in Wernicke's disease." The justification for such a conclusion can suitably be questioned, since vascular changes of this type are commonly seen as a reaction to tissue necrosis of varying etiology.

Swank and Prados[11] have made careful observations on the behavior of both acutely and chronically thiamine-deficient pigeons,

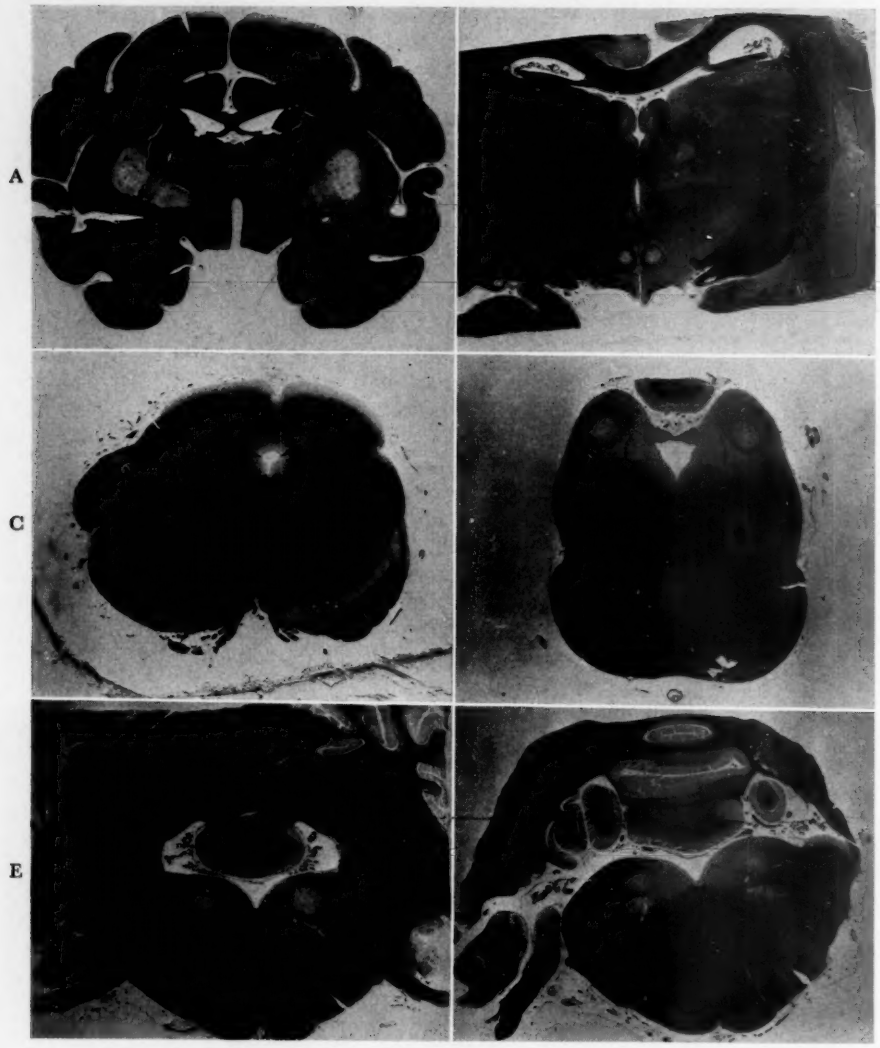

FIG. 3. The effect of thiamine deficiency on the brain of the monkey. These photographs have been prepared from sections kindly supplied by Rinehart et al.[12] A, Coronal section showing symmetrical areas of pannecrosis in the lenticular nucleus. Loyez stain. B, Coronal section through the diencephalon, showing the lesions in the mammillary bodies and habenular nuclei. Loyez stain. C, Symmetrical lesions within the third nerve nuclei. Loyez stain. D, Symmetrical lesions in the inferior colliculi. Loyez stain. E, Symmetrical lesions in the main vestibular nuclei. Loyez stain. F, Symmetrical lesions in the area of the floor of the fourth ventricle.

and have attempted to relate the clinical to the pathologic findings. Acute deficiency resulted in the abrupt development of opisthotonos, preceded at times by rhythmic lateral rotation and extensor thrusts of the neck; no pathologic alterations were observed in such animals. The chronic deficiency state was characterized mainly by staggering and by weakness of the limbs. In these animals the peripheral and central terminations of the vestibular nerves, as well as many basket terminations and mossy fibers in the cerebel-

lum, were said to be degenerated. "Brain hemorrhages" were considered to be a secondary phenomenon, unrelated to the clinical behavior.

Although the athiaminotic state described by Swank and Prados, was produced by the use of a purified diet containing autoclaved yeast, the addition of thiamine alone brought about a prompt reversal of symptoms. Furthermore, it was claimed that the damaged peripheral and cranial nerves would regenerate with the administration of thiamine. The pathologic changes described by these authors are difficult to evaluate. The alterations themselves are minor and the authors' observations are based entirely on the use of silver stains, which are unfortunately subject to many artefacts. Furthermore, the reports do not include any description of neuronal changes, glial reactions and the state of the myelinated fibers, using standard neuropathologic methods.

MONKEYS

Rinehart et al.[12] have described the effect of experimental thiamine deficiency on the nervous system of the *Rhesus monkey*. They used a purified diet to which all vitamins, except thiamine, were added. Clinically the animals manifested progressive apathy, inactivity and weakness of the limbs. This was followed by ataxia, tremulousness and occasionally by retching and bilateral ptosis. The administration of thiamine, even in advanced depletion, was followed by dramatic improvement of symptoms.

The brains of these animals showed bilaterally symmetrical lesions in the putamen and globus pallidus, the mammillary body, thalamus, hypothalamus, superior and inferior colliculus, the main vestibular, oculomotor and vagal nucleus, and the cerebellar cortex (Fig. 3). Essentially, the lesions took the form of sharply defined areas of tissue necrosis in which all parenchymal elements were destroyed to a varying degree, the nerve cells being relatively resistant. Dilated, tortuous capillaries with some endothelial proliferation were seen in the lesions, but no hemorrhages. The histopathologic changes in the monkeys closely resemble those of Wernicke's disease in man. The distribution of the lesions was more widespread in the monkey than in man insofar as the lenticular nuclei were involved in the former; apart from this, however, the topography was strikingly similar.

CHASTEK PARALYSIS

In 1932, a specific dietary disease of silver foxes was first observed in Minnesota, on the ranch of J. S. Chastek. This syndrome, now generally known as Chastek paralysis, occurred when raw fish was added to the diet. After three to six weeks of such a diet, the animal went "off feed," and sequentially ataxia, paralysis of the limbs, an abnormal sensitivity to pain and convulsions developed and the animal finally died. Ten years after the original description of this disease, Krampnitz and Wooley[13] discovered that a thiamine-splitting enzyme, thiaminase, contained in the raw fish viscera, was the causative agent.

The pathologic changes in Chastek paralysis, as reported by Evans et al.,[14] consisted of bilaterally symmetrical lesions in the dorsal motor nucleus of the vagus, the medial vestibular, gracile, cuneate, inferior olivary and oculomotor nucleus, as well as in the inferior colliculus and the folia of the cerebellar vermis. Less frequently, lesions occurred in the other vestibular nuclei, hypoglossal nuclei, superior colliculi and the thalamus. According to these authors, a fully developed lesion appeared on a hyperemic area in which there was an apparent increase in the number of small vessels, which were very cellular and irregularly shaped. They believed that the numerous small parenchymal hemorrhages resulted from the degenerative vascular changes, which they regarded as the primary pathologic changes.

Recently, an illness clinically and etiologically similar to Chastek paralysis has been described in *cats* fed a diet of commercially canned cat food containing whole fish.[15] It is likely that this deficiency state was brought about in the same manner as Chastek paralysis. Apparently the thiaminase in the fish inactivated the thiamine in the fish-cereal mixture, which had been allowed to stand before exposure to processing temperature.

FIG. 4. Section through the brain stem of a thiamine-deficient cat, from material supplied by Jubb et al.[15] Low power view showing a lesion in the floor of the fourth ventricle.

The lesions described by Jubb et al.[15] were confined to the central nervous system and on gross inspection consisted of bilaterally symmetrical hemorrhages in the periventricular gray matter. These were most consistently found in the inferior colliculi, less frequently in the vestibular, oculomotor and other brain stem nuclei, and only irregularly in the mammillary bodies and superior colliculi. Microscopically, these authors described lesions of two main types: (1) varicose dilatation of the blood vessels with focal extravasations and (2) foci consisting of vascular hypertrophy, gliosis and "gitter" cell formation. The former lesion was considered to be typical of this disease, and the latter was interpreted as reparative in nature. Jubb and Saunders permitted us to examine some of their pathologic material. We were impressed with the sharply defined, bilaterally symmetrical areas of tissue necrosis, characterized by a loss of architecture, prominence of capillary endothelial cells, macrophage activity and astrocytic proliferation (Figs. 4 and 5). These lesions are very much like those described in the rat, pigeon and monkey. We would be inclined to regard these lesions, rather than the focal hemorrhages, as the more significant ones, for reasons that have been discussed in relation to the lesions in pigeons.

BIOCHEMICAL ASPECTS OF THIAMINE DEFICIENCY

Before considering the aberrations of cerebral metabolism engendered by the dietary lack of thiamine, the state of our knowledge concerning the biochemical role of this vitamin will be briefly summarized.

Most of the naturally occurring thiamine in vegetable foods exists in the free, non-phosphorylated form; in meats, however, cocarboxylase or thiamine diphosphate predominates. Ingested thiamine must be phosphorylated in order to become metabolically active as a cofactor.[16] Phosphorylation is probably accomplished by a group of enzymes which may be referred to as thiaminephosphokinases. Such enzymes are most active in the liver, but are also found in the brain and other tissues. Presumably phosphorylated thiamine is less diffusible than free thiamine, and in order to facilitate diffusion within tissues, the phosphate linkage must first be split off. This is accomplished by pyrophosphatases of various types which are present in mammalian brain.[17]

Thiaminases are a group of enzymes capable of splitting the thiamine molecule into two parts, a pyrimidine and a thiazole moiety. Thiaminase has been found in a variety of animal tissues, as well as in bacteria and certain fern species.[18]

Cocarboxylase acts as an essential cofactor in three separate steps of carbohydrate metabolism. The first of these occurs in the Embden-Meyerhoff glycolytic pathway: thiamine catalyzes the decarboxylation of pyruvic acid to acetyl coenzyme A. This step has been found to require diphosphopyridine nucleotide and lipoic acid. The latter is a biocatalyst of known structure, biologically active in extremely small amounts. In bacteria lipoic acid forms a complex known as lipothiamide, necessary in the oxidative decarboxylation of keto acids. Lipoic acid, however, has not

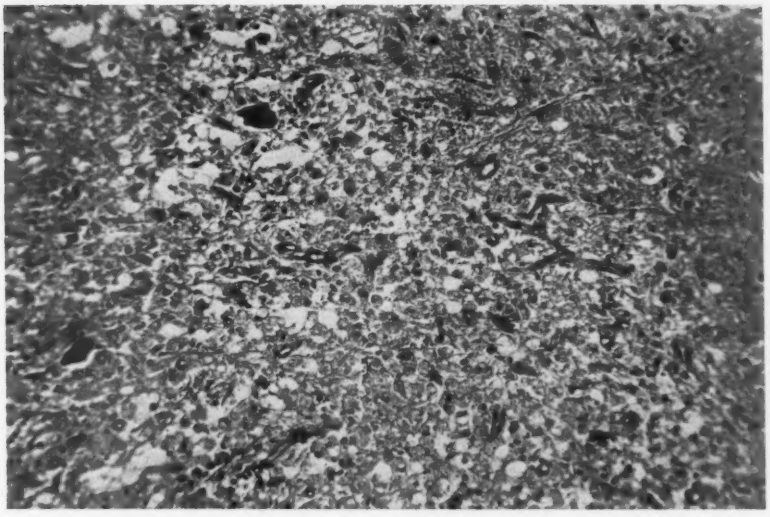

FIG. 5. High power view of Figure 4 showing necrosis of tissue, proliferation of blood vessels and glial activity.

yet been demonstrated to be a dietary requirement for higher animals.[19]

The second thiamine-dependent step is entirely analogous to the first, and involves the decarboxylation of α ketoglutarate to succinyl coenzyme A in the citric acid cycle. The third step occurs in the pentose phosphate pathway or hexosemonophosphate shunt, an oxidative pathway of importance in the synthesis of nucleic acid pentose. The quantitative utilization of carbohydrate by means of this series of reactions in brain has not yet been fully evaluated.

Recently, a thiamine-dependent enzyme, transketolase, has been demonstrated in the pentose pathway.[20] This enzyme has been found in yeast, spinach and in the cytoplasm of animal cells including those of the brain. Transketolase acts as a catalyst in the following reactions:

(1) Ribose-5-phosphate + xylulose-5-phosphate $\rightleftharpoons$ sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate

(2) Xylulose-5-phosphate + erythrose-4-phosphate $\rightleftharpoons$ fructose-6-phosphate + glyceraldehyde-3-phosphate

In experimental animals thiamine deficiency is shown to produce major disturbances in cerebral metabolism. These abnormalities have come to be known as the "biochemical lesion," a term originally introduced by Peters.[21] The original observations concerning this concept were made by Kinnersley and Peters,[22] who were able to correlate symptoms of thiamine deficiency with cerebral lactic acid levels. They demonstrated that lactic acid accumulated in the brains of athiaminotic pigeons before signs of avitaminosis were apparent. This accumulation occurred mainly in the lower brain stem in contrast to the cerebrum, cerebellum optic lobes and mid brain. Other conditions known to increase cerebral lactic acid levels, such as exercise, caused an evenly distributed rise in the various portions of the brain, suggesting that the chemical abnormality in thiamine deficiency was of localized character. Further investigations by Meiklejohn, Passmore and Peters[23] revealed that the oxygen uptake by thiamine-deficient pigeon brain was markedly depressed. This depression closely paralleled the severity of the deficiency state and was particularly marked when pyruvate was used as substrate. This demonstrated an impairment of pyruvate metabolism in thiamine deficiency.

If minced brain or brain dispersions were used in these experiments, the impairment of pyruvate metabolism in oxygen uptake could be reversed by the addition of cocarboxylase to the *in vitro* system.[24] In contrast, if brain slices were used, the *in vitro* addition of thiamine itself or its pyrophosphoric ester brought about an increased oxygen uptake. In the latter experiments, plain thiamine proved more effective than cocarboxylase, suggesting that cocarboxylase diffuses less readily into intact cells, and that cerebral tissue is capable of phosphorylating thiamine. In the experiments using minced or brain dispersions, the *in vitro* addition of thiamine itself was ineffective in correcting the disorder of pyruvate metabolism, whereas the opposite occurred with the *in vivo* addition of the vitamin.

These observations prove that the active form of vitamin B_1 in animal tissues is its pyrophosphoric ester, an idea originally put forth by Lohmann and Schuster.[25] Further work by Ochoa and Peters[26] showed that the liver was primarily responsible for the phosphorylation of thiamine, and that the brain and heart require more thiamine than do other organs. Thus one dose of vitamin B_1 administered to severely thiamine deficient pigeons caused an immediate increase in liver cocarboxylase levels although the brain cocarboxylase remained low. Small doses of thiamine administered daily for three days resulted in a decrease of liver cocarboxylase and a preferential increase in the brain and the heart, the two organs most affected by a deficiency of vitamin B_1.

TABLE I

Distribution of Total Thiamine in Normal Rat Brain, Spinal Cord and Sciatic Nerve
(micrograms of thiamine per gram dry weight)

Area Sampled	Thiamine Content	
	Average (μg./gm.)	Standard Deviation
Cortex	13.0	±2.5
Caudate nucleus	17.4	±4.5
Thalamus	9.2	±2.3
Hypothalamus	13.0	±3.2
Mammillary region	12.4	±2.4
Periaqueductal region	13.2	±3.5
Lateral pontine tegmentum	11.5	±4.7
Base of pons	13.1	±2.5
Vermis	21.1	±4.2
Medulla	13.4	±3.1
Total Brain	13.7	±1.2
Spinal cord	8.4	±2.4
Sciatic nerve	4.1	±0.9

TABLE II

Comparative Brain Thiamine Content

Area Sampled	Micrograms per Gram Dry Weight		
	Rat	Bovine	Human
Cerebrum			
Gray matter	13.0	6.4	8.7
White matter	...	..	3.4
Cerebellum	21.1	5.3	9.7
Mammillary body	12.4	5.2	9.3
Spinal cord			
Gray matter	12.1	..	..
White matter	2.8	..	..

LOCALIZED LESIONS

Reference has already been made to the fact that thiamine deprivation in animals bears a resemblance pathologically to Wernicke's disease in man. The histopathologic process selects, in a reproducible and symmetrical fashion, certain parts of the nervous system which appear to be selectively vulnerable to a lack of thiamine. It is also known that in experimental animals a "biochemical lesion" can be related to the development of signs and symptoms of deficiency and that the brain as a whole becomes depleted of thiamine. One possible explanation of this selective vulnerability was thought to be a difference in thiamine content between the affected and unaffected portions of the brain, and perhaps another a differential rate or extent of thiamine depletion in the course of vitamin deprivation.

As a first step in our investigations, it seemed essential to determine the distribution of total thiamine (free and phosphorylated) in various parts of the nervous systems of both normal and thiamine deficient rats in various stages

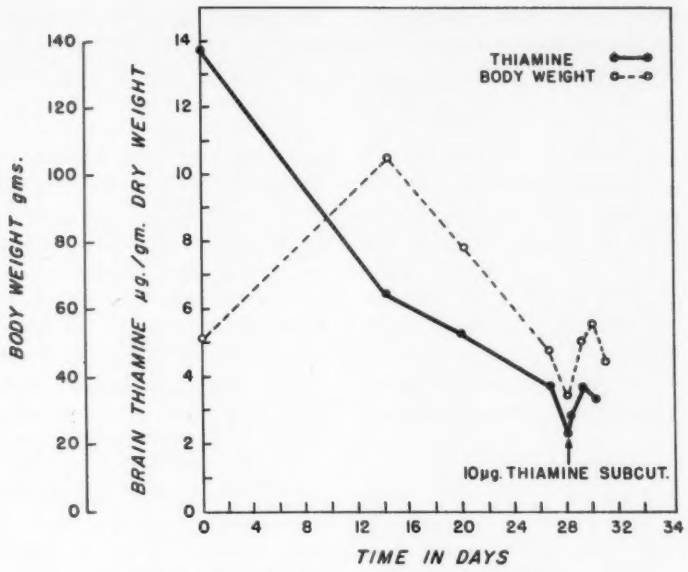

FIG. 6. Brain thiamine in progressive depletion.

of depletion and replenishment. This necessitated the adaptation of existing methods for the quantitative estimation of thiamine in small samples of tissue. A summary of this method, the details of which have been previously reported,[27] follows:

Total thiamine was estimated in ten representative parts of normal and deficient rat brain, spinal cord and peripheral nerves. Fresh-frozen brain was cut into coronal slices 2 to 3 mm. thick. Cylindrical pieces of tissue from different regions were removed from each slice by means of a metal corkborer. The cylinder thus obtained was then cut serially into sections, each of which had a diameter of 2 mm., a thickness of 40 μ and a dry weight of 25 to 35 μg. Sections were used for histologic control, dry weight and thiamine determinations. All thiamine analyses were carried out in triplicate by means of a sensitive microfluorometric thiochrome method.

The results of total thiamine determination of selected areas of the neuraxis of eight normal animals are summarized on Table I.

Thiamine values are expressed in micrograms per gram dry weight. The results disclose that the distribution of total thiamine in the brains of normal rats is relatively uniform, with certain exceptions: the cerebellar vermis is significantly higher in its vitamin content, the thalamus is lower, and the lateral pontine tegmentum, site of the most severe histopathologic alterations, slightly lower than the remainder of the brain. When rat brain was compared with human brain, it was found in both species that total thiamine content was highest in the cerebellum (Table II). This observation has been confirmed by at least one investigator.[28] Experimental evidence suggests that the cerebellum possesses relatively high metabolic activity: oxygen uptake, cytochrome oxidase activity, nucleotide concentration, especially adenosine triphosphate are higher in the cerebellum than in other portions of the brain. The cellular composition of the cerebellum may account for its higher metabolic rate. The lower levels of thiamine found in the thalamus may reflect admixture of white matter in the samples, the latter being relatively low in thiamine content.

Similar measurements were then carried out on twelve animals sacrificed at various stages of depletion and replenishment. The rate and extent of depletion was noted to be roughly the same in all parts of the brain. No specific pattern was evident. Figure 6 illustrates the

relationship between the clinical behavior of the animals on the one hand, and a composite thiamine value for all ten areas of the brain on the other. Time (in days) on the deficient diet is shown by the abscissa. Total brain thiamine can be reduced to 50 per cent of normal before any clinical signs of deficiency become manifest. A further decrease to 30 per cent of normal is accompanied by slowness and unsteadiness of gait. When thiamine content in the brain is reduced to less than 20 per cent of normal, a severe disturbance of posture and equilibrium occurs, as well as irreversible pathologic changes in some animals. If, at this point, 10 μg. of thiamine is given subcutaneously, the level again rises to 30 per cent of normal, and symptoms are dramatically improved. After forty-eight hours the thiamine level again falls to less than 30 per cent, although no worsening of symptoms can be noted.

From these experiments, it must be concluded that the selective physiologic and pathologic vulnerability of certain parts of the brain cannot be correlated with either the vitamin content of these parts, or the rate at which this vitamin decreases during induced deficiency. It is apparent, however, that the brain contains a great excess of thiamine and that the level below which nervous tissue is irreparably damaged is extremely low. This level may differ from one experimental animal to another and may account for the unpredictable production of lesions. It is possible that our sampling methods are too crude and that determinations on isolated cells would indeed show differences in thiamine content.

These experiments supply data concerning thiamine as a coenzyme of the alpha-keto oxidases and transketolase systems; however, they fail to contribute information regarding the apoenzyme part of these enzyme systems. A constant ratio of coenzyme to apoenzyme may well be of importance in maintaining the integrity of the nervous system. Selective vulnerability could perhaps be explained by an imbalance of this ratio in those parts of the brain most readily affected by thiamine deficiency.

CONCLUSION

The clinical and pathologic features of experimentally-induced thiamine deficiency in animals, as they pertain to the central nervous system, are reviewed. The findings in the deficient rat, pigeon, monkey, fox and cat are being compared with one another as well as with our own findings in experimental thiamine deficiency states and in human Wernicke's disease. The lesions in these animal species are remarkably similar to each other and also bear a striking similarity with their human counterpart. In all these species the fundamental lesion in thiamine deprivation is a symmetrical focal pannecrosis affecting the nuclei of the brain stem and diencephalon. The occurrence of hemorrhages are probably a secondary phenomenon.

The role of thiamine and its pyrophosphoric ester, cocarboxylase, in metabolism of the central nervous system is also summarized, and attention is drawn to the so-called "biochemical lesion" which antedates the symptoms of deficiency. The results of our own experiments concerning the total thiamine content in different parts of the central nervous system in normal and thiamine-deficient rats at various stages of depletion and replenishment are presented.

Although the experimental data have not provided a solution to the fundamental problem of why certain selected portions of the brain are involved in thiamine deficiency, they serve as a point of departure for further investigations on the effects of thiamine deficiency in the nervous system.

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DISCUSSION

DR. JOSEPH M. FOLEY (*Jersey City, New Jersey*): Emerging from the clinical, pathologic and experimental data that have been presented are some very practical considerations. Actually, a large body of data that can be employed effectively in the prevention and treatment of many of the complications of alcoholism are already assembled.

However, I think you have also gained the impression, as I have, that much is still to be learned about alcohol and its effect on the nervous system. In studying this most important area of clinical and chemical investigation, we probably will learn not only about alcohol in the nervous system but also a great deal more about toxic and nutritional disorders, diseases of the central nervous system itself and the general capacity of the organism under many biological situations.

The Intermediary Metabolism of Alcohol

W. W. WESTERFELD, PH.D.*

THE probable metabolic pathway for alcohol (Fig. 1) involves an initial oxidation to acetaldehyde, a conversion of the latter to acetyl co-enzyme A (CoA) either directly or via acetic acid, and the complete combustion of acetyl CoA in the citric acid cycle. The evidence for this pathway is reasonably substantial, and no data have been obtained so far which cast any serious doubt upon it.

ALCOHOL TO ACETALDEHYDE

The enzyme which carries out the oxidation of alcohol to acetaldehyde, alcohol dehydrogenase, has been studied extensively *in vitro*, and the appearance of acetaldehyde in body fluids during alchohol metabolism demonstrates that this reaction takes place *in vivo*. This enzyme is localized within the liver and kidney, and most of the oxidation of alcohol *in vivo* takes place in the liver. Catalase plus hydrogen peroxide are capable of oxidizing alcohol to acetaldehyde *in vitro*, but this has no metabolic significance.[1, 2] The metabolic removal of acetaldehyde is faster than its formation from alcohol, so this initial oxidation of alcohol is the rate-limiting reaction in its over-all removal. Since this reaction is not influenced by the over-all metabolic needs of the organism, the rate of alcohol metabolism is not affected by hyperthyroidism, exposure to cold or muscular exercise.

Alcohol dehydrogenase is a zinc-containing enzyme[3,4] which utilizes DPN as the hydrogen acceptor, and is dependent upon free SH groups in the protein for activity.[5,6] The zinc is one of the binding sites of DPN with the enzyme.[7, 8] The liver enzyme has a molecular weight of 84,000, contains 2 atoms of zinc and 2 molecules of bound DPN[9]; the yeast alcohol dehydrogenase is approximately twice as large and contains 4 or 5 atoms of zinc plus an equal number of DPN units. The equilibrium for the oxidation of alcohol to acetaldehyde *in vitro* is markedly in favor of the alcohol, and the reaction stops when relatively little acetaldehyde has been formed unless the latter is removed from the sphere of action. The DPNH formed in this reaction must also be removed in order to allow the reaction to proceed.

In an isolated system the oxidation-reduction (OR) potential for the DPN:DPNH system is in the neighborhood of −0.3 volts. However, when this system combines with the alcohol dehydrogenase at a pH of 7 to 7.4, the OR potential increases to −0.20 to −0.22 volts.[10] The potential usually given for the ethanol:acetaldehyde system is approximately −0.16 volts. Hence the alcohol is being oxidized by a system of lower potential. This is not unique for alcohol. Most substrate systems which are oxidized by DPN or TPN enzymes appear to have higher potentials than the oxidant system. This undoubtedly provides a good mechanism for controlling the rate of oxidation of the substrate and preventing its explosive combustion. However, such conditions are unfavorable for the oxidation of alcohol in an isolated system, and can be utilized biologically only because the products of the reaction (acetaldehyde and DPNH) are removed continuously.

The oxidation of alcohol by the alcohol dehydrogenase-DPN system theoretically and actually occurs at a low ratio of acetaldehyde:-alcohol. Liver normally contains a DPN:-DPNH ratio of 1.7 to 1.8:1.[11,12] During the

From the Department of Biochemistry, State University of New York, Upstate Medical Center, Syracuse, New York.

* Professor of Biochemistry.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

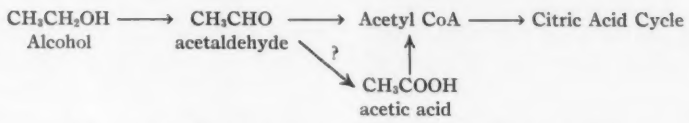

FIG. 1. The probable pathway for the metabolism of alcohol.

metabolism of alcohol the DPN:DPNH ratio falls to 1 to 1.2:1,[11,12] and the corresponding acetaldehyde:alcohol ratio is about 1:40. This ratio is considerably higher than the 1:500 ratio found by Lundquist et al.[13] for liver homogenates, and suggests that the OR potentials of the two systems are further apart than indicated. Irrespective of the exact values, it is evident that the oxidation of alcohol stops when relatively little acetaldehyde has been formed. Alcohol could be a relatively effective substrate for providing a more reduced atmosphere in the animal body since it requires no pretreatment to be a hydrogen donor, and no controlling mechanism could be interposed to prevent its acting as a hydrogen donor.

A consideration of OR potentials as they apply to an equilibrium reaction *in vitro* cannot provide any information about reaction rates *in vivo*. Alcohol seems to be metabolized as rapidly in the presence of the high levels of acetaldehyde caused by Antabuse® as in the presence of the relatively low levels of acetaldehyde found in the absence of Antabuse. A more important factor in determining the rate of alcohol metabolism is the rate at which DPNH is reoxidized to DPN.[12]

There are potentially two methods by which DPNH can be reoxidized to DPN. One is by the classic electron transport chain: the flavoprotein-cytochrome system. This chain is localized in the mitochondria and seems to be responsible for the bulk of the oxygen utilization in the body irrespective of the substrates being oxidized. Alcohol dehydrogenase is present in the cytoplasm (soluble supernatant fraction) of the cell, and the DPNH which is formed during alcohol metabolism must therefore be transported to the mitochondria before being oxidized. This would seem to represent another biological means of slowing down and controlling a metabolic oxidation. Alcohol cannot be oxidized any faster than the DPNH is regenerated, and the DPN carrier must shuttle back and forth between the electron supply and the electron furnace. Again, this situation is not unique for alcohol since other substrates are similarly oxidized in the cytoplasm by DPN or TPN enzymes. While the total amount of DPN:DPNH could theoretically be limiting for this reaction, the rate of alcohol metabolism is not decreased by a niacin deficiency[14] or increased by the higher levels of DPN resulting from the administration of nicotinamide.[15]

DPNH can also be oxidized by using some electron acceptor other than oxygen, i.e., some other substrate. The addition of pyruvate to an anaerobic liver homogenate reoxidizes the DPNH.[13] In this type of reaction the hydrogen is removed from the alcohol and passed by way of DPN to another substrate, such as pyruvate. Such a coupled oxidation-reduction reaction can take place because the DPN carrier is required for both the alcohol-acetaldehyde and the pyruvate-lactate interconversions; the DPN can therefore act as a shuttle between the two substrate systems. This coupled oxidation-reduction reaction would cause the accumulation of another substrate (such as lactate), and would therefore tend to be self-limiting. However, it provides a possible means whereby the oxidizing capacity of other tissues could be borrowed by the liver. Any lactate formed by this type of reaction which escaped from the liver to the muscles would be reoxidized to pyruvate at that site. In effect the liver would be oxidizing alcohol by utilizing lactate as a carrier of hydrogen to the cytochrome chain in the muscles. A similar use of the dihydroxyacetone phosphate-α-glycerophosphate system could transfer hydrogens from the cytoplasmic DPNH to the mitochondrial chain within the liver cell itself.

There is another type of coupled oxidation-reduction which does not have the same self-limitation as the utilization of the lactate-

pyruvate system. This is the synthesis of fat. The formation of a fatty acid from acetyl CoA is a reductive process, and the hydrogens used in this process must come from the dehydrogenation of other substrates. In this coupled reaction the reduction product (newly synthesized fatty acid) can accumulate without limit because it is deposited as neutral fat in an open-ended system.

It is not known how extensively fat synthesis utilizes the hydrogens derived from the oxidation of alcohol. The hydrogens from both DPNH and TPNH are utilized at different points in the synthesis of fatty acids. However, the amount of TPNH available seems to be the limiting factor in this process. Excessive amounts of DPNH could not automatically lead to increased fat synthesis unless (1) an adequate supply of TPNH were available simultaneously from the oxidation of some suitable TPNH-requiring substrate, or (2) the liver transhydrogenase shifts some of the hydrogens from the DPN to the TPN system.

A number of substances have been reported to speed the rate of alcohol metabolism. These substances include insulin plus glucose,[16] fructose,[17] pyruvate, and alanine.[12] This area remains controversial[18,19] for reasons which are not entirely clear. Positive effects have been obtained most consistently when the initial rate of alcohol removal was slow, but small increases have also been observed when the initial rate was relatively high. Since the oxidation of alcohol to acetaldehyde is the rate-limiting reaction in alcohol metabolism, the effect of these substances must be on this reaction. The large daily variation in the rate of alcohol metabolism in the same animal[20] strongly suggests that some factor other than the concentration of alcohol dehydrogenase is involved in determining the rate of this reaction. The only mechanism which is apparent at present is through some influence on the reoxidation of DPNH.[12] This could involve a coupled oxidation-reduction reaction between alcohol and some substrate such as pyruvate or the coupled oxidation-reduction reactions involved in fat synthesis. Moreover, insulin and fructose could stimulate the pentose pathway of carbohydrate metabolism and thereby provide additional TPNH for increased fat synthesis. This might also explain why alcohol metabolism causes a simultaneous disappearance of carbohydrate.

ACETALDEHYDE METABOLISM

The metabolic fate of acetaldehyde has not been clearly established. Like alcohol, it is metabolized primarily in the liver. There are at least two molybdenum-containing (Mo) enzymes (xanthine oxidase and aldehyde oxidase) and one DPN-enzyme present in liver which can oxidize acetaldehyde to acetic acid *in vitro*, but the Mo-enzymes are relatively unimportant in this process.[21] Liver slices, homogenates and rat liver mitochondria all oxidize acetaldehyde to acetic acid by means of the nonspecific DPN-requiring aldehyde dehydrogenase.[13,22] This enzyme is inhibited by Antabuse, and might be expected to carry out the oxidation of acetaldehyde to acetic acid *in vivo*. However 90 per cent of this aldehyde dehydrogenase activity can be removed from rat liver by feeding the animals low protein diet; this depletion has relatively little effect on the metabolism of acetaldehyde *in vivo*. In the dog the feeding of a low protein diet has no effect on the metabolism of acetaldehyde. Hence there is some doubt about the role of this enzyme in acetaldehyde metabolism in the intact animal. There is actually no evidence that free acetic acid is formed during the course of alcohol or acetaldehyde metabolism *in vivo*, and it is possible that acetaldehyde is converted to acetyl CoA by a more direct process which bypasses acetic acid.

Acetyl CoA is considered to be an intermediate in alcohol metabolism for two reasons: (1) there is no other known pathway for the metabolism of this type of 2-carbon unit, and (2) isotopic alcohol is found in the acetyl group of a metabolically acetylated amine. The latter point would be conclusive evidence for conversion to acetyl CoA if it were also certain that acetyl CoA is the only acetylating agent in the body; it is the only one known at the present time.

Animal tissues contain other enzymes which are capable of utilizing acetaldehyde as a substrate and forming products other than acetate or the acetyl CoA unit. These include (1) different aldolases which can combine acetaldehyde with dihydroxyacetone phosphate or glyceraldehyde phosphate to form pentose derivatives'[23] (2) carboxylase which can combine acetaldehyde with a decarboxylated pyruvate to form acetoin, and (3) an aldolase which condenses acetaldehyde with glycine to form threonine.[24] To date there is no evidence to suggest that any of these potential pathways is utilized significantly in the metabolism of acetaldehyde; there is good evidence that the threonine[24] and acetoin pathways are not used *in vivo*, and none of these condensation reactions take place in an anaerobic liver homogenate metabolizing acetaldehyde.[13]

Acetaldehyde is not a major metabolite in the breakdown of the usual foodstuffs. Small amounts of acetaldehyde and alcohol apparently arise normally from pyruvate[25] and some acetaldehyde might be formed from the β-alanine produced as an intermediate in pyrimidine degradation.[26] But from a quantitative standpoint, alcohol and acetaldehyde are relatively unique to alcohol metabolism. Antabuse produces a relatively specific block in acetaldehyde metabolism and exhibits an effect only during alcohol metabolism. Both the alcohol and the acetaldehyde are metabolized in the presence of Antabuse, but the process takes place at higher concentrations of blood acetaldehyde than would otherwise obtain.[27]

ACETYL CoA METABOLISM

There are quantitatively several major and several minor pathways for the disposal or utilization of acetyl CoA. The major oxidative pathway is the citric acid cycle. It is by means of this cycle that the alcohol is completely burned to CO_2 and H_2O. It is also by means of this cycle that traces of the alcohol carbon atoms are believed to be incorporated into proteins and carbohydrates.

Acetyl CoA is the starting point for the synthesis of fatty acids and cholesterol. Quantitatively the former is without limit, while the total amount of cholesterol synthesized per day is circumscribed. Alcohol, acetaldehyde and acetic acid are all good precursors of fatty acids and cholesterol, presumably because they are metabolized via acetyl CoA. Alcohol and acetaldehyde appear to be better precursors than acetic acid, but the reason for this is not yet known and might be artifactual due to experimental technics.[28,29]

In diabetes, the acetyl CoA units are formed from fatty acid degradation in the liver faster than they can be metabolized, and they accumulate as ketone bodies. Acetoacetate can also be formed from the acetyl CoA units derived from alcohol, and more ketones are produced from alcohol by a glycogen-poor (fasted) than by a glycogen-rich liver.[11] There is also less accumulation of liver lipid following the administration of alcohol to fasted rate than to well fed rats.[30] Ketone bodies do not accumulate in the normal animal metabolizing alcohol, and no extra ketones would be expected in the diabetic animal if the alcohol simply replaced fatty acids as the source of the acetyl CoA units.

The acetoacetate formed by the liver can normally be metabolized by muscle and other tissues at a rate which prevents its accumulation. The conversion of alcohol to acetoacetate provides a mechanism whereby alcohol can contribute energy to those tissues (e.g., muscle) which are incapable of utilizing alcohol itself. Some such relationship would seem to be essential in those situations in which alcohol contributes the bulk of the daily calories.

STUDIES IN INTACT ANIMALS

While most metabolic reactions are studied and developed in *in vitro* system, such studies merely demonstrate that a given reaction is possible. They must be supplemented with studies in the intact animal in order to prove that a given reaction or a postulated sequence of reactions actually does take place.

The relatively few such studies available in the alcohol field at present are consistent with the postulated pathway. They show

essentially that (1) alcohol and acetate metabolism are parallel, i.e., the 2-carbon unit from alcohol is handled by the body in essentially the same way as the 2-carbon unit from acetate;[31] and (2) the distribution of the alcohol carbons found in glycerol and glycogen is consistent with the passage of acetyl CoA through the citric acid cycle.[32]

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Alcohol-Induced Triglyceride Deposition in Liver Through Derangement of Fat Transport

B. B. BRODIE, PH.D.,* W. M. BUTLER, JR., M.S.,† M. G. HORNING, PH.D.,‡
R. P. MAICKEL, PH.D.§ AND H. M. MALING, PH.D.||

THE hypothalamic-pituitary system controls the activity of a number of biochemical functions. One such function is fat transport, which ordinarily responds to the energy requirements of the body by mobilizing energy-rich fatty acids from adipose tissue and transporting them to various organs.

Many substances can interfere with the endocrine control of the fat transport system. Among these is alcohol. Our studies show that large doses of alcohol in rats can upset the pituitary control of fat transport. As a result excessive amounts of triglycerides are mobilized from adipose tissue as free fatty acids; these are carried by plasma to liver, reformed to triglycerides, and deposited as such in this organ.

Our studies on the effect of alcohol on fat transport have been facilitated by the development of a simple direct method for the estimation of liver triglycerides.[1] This method lacks the considerable error of other procedures in which the triglyceride value is calculated from total lipid, less the values for cholesterol, cholesterol esters and phospholipids.

LIVER TRIGLYCERIDE

The effect of single doses of orally administered alcohol on the liver triglyceride content of female rats was determined. Eighteen hours after the administration of 4.8 gm./kg. of alcohol, the triglyceride level had risen almost threefold (Table 1). The level was maximal in fifteen to twenty hours and returned to normal in thirty to fifty hours. Pathologic examination disclosed little or no necrosis. Larger doses of alcohol produced a greater rise in triglycerides; a dose of 6 gm./kg. increased the triglycerides almost fivefold (Table 1). An increase in triglycerides after alcohol has also been reported by di Luzio,[2] and an increase in total lipids has been found by Mallov and Bloch.[3] We found no change in the liver phospholipids, a finding in agreement with that of di Luzio.[2]

The problem arises whether the fat deposition results from (1) a stimulatory effect of alcohol on fatty acid synthesis in the liver, as suggested by Lieber et al.[4]; (2) an increased rate of fatty acid formation by incorporation of alcohol or a two-carbon fragment that it forms[5]; (3) inhibition of fat metabolism in liver; or (4) mobilization of fatty acids from the triglycerides in adipose tissue.

TABLE 1
Liver Triglyceride Levels After Single Large Oral Doses of Alcohol in Female Rats

Treatment	Liver Triglycerides (mg./gm. ± S.E.)
Saline (12.0 ml./kg. orally)....	4.8 ± 0.25 (4)
Alcohol (4.8 gm./kg.).....	12.7 ± 1.7 (9)
Alcohol (6.0 gm./kg.).....	22.6 ± 2.9 (8)

NOTE: Alcohol, in a 50 per cent solution by volume, was administered to NIH female Sprague-Dawley rats weighing 150 to 200 gm. that were killed eighteen hours later. Figures in parentheses represent number of animals.

From the Laboratory of Chemical Pharmacology and Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland.

* Chief, Laboratory of Chemical Pharmacology; † Chemist, Laboratory of Chemical Pharmacology; Present address: Hazelton Laboratories, Falls Church, Virginia; ‡ Biochemist, Laboratory of Cellular Physiology and Metabolism; § Biochemist, Laboratory of Pharmacology; || Head, Section on Physiology, Laboratory of Chemical Pharmacology.

Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of The National Vitamin Foundation, Inc., March 1, 1960.

TABLE II

The Linoleic Acid and Oleic Acid Content of Triglycerides Deposited in Liver and of Triglycerides in Adipose Tissue Eighteen Hours After Oral Administration of Alcohol (4.8 gm./kg.) to Rats

Tissue	Treatment	No. of Rats	Liver Triglycerides (mg./gm. ± S.E.)	Linoleic Acid		Oleic Acid	
				In Total Liver Triglycerides (% ± S.E.)	In Deposited Liver Triglycerides (% ± S.E.)	In Total Liver Triglycerides (% ± S.E.)	In Deposited Liver Triglycerides (% ± S.E.)
Liver.....	Control	6	6.5 ± 1.8	29.2 ± 1.2	...	29.3 ± 1.3	...
Liver.....	Alcohol	6	24.2 ± 5.7	25.8 ± 0.5	23.7 ± 1.1	34.6 ± 0.9	37.5 ± 1.7
Adipose tissue.....	...	10	...	21.2 ± 0.3	...	38.1 ± 0.5	...

NOTE: The linoleic acid in the triglycerides deposited in liver was calculated for each rat from the following equation:

$$y = \frac{AB - (6.5)(29.2)}{A - 6.5}$$

where A = total liver triglycerides in milligram per gram after alcohol; B = per cent linoleic acid in total liver triglycerides after alcohol; and y = per cent linoleic acid in deposited liver triglycerides.

A direct answer to this problem has been provided by the application of gas chromatography to the assay of linoleic acid in liver triglycerides. This unsaturated fatty acid is not synthesized by the rat, but is of dietary origin. The liver triglycerides formed by the action of the alcohol had virtually the same content of linoleic acid as the triglycerides of adipose tissue (Table II). This suggested that the triglycerides were formed from fatty acids mobilized from adipose tissue. As further evidence of this view was the finding that the oleic acid content of the triglycerides formed in liver was also almost identical with that of adipose tissue (Table II). Since the concentration of both acids in the liver reflects that in adipose tissue, little if any of the triglycerides deposited in liver could have been derived from an increased synthesis of fatty acids in this organ.

To verify the assumption that linoleic acid was not formed in the body in these experiments, adipose tissue was labeled by pretreatment of rats with C^{14}-acetate, twenty-four hours before giving the alcohol. All the fatty acids in liver triglycerides, except linoleic acid, were found to be highly labeled. This demonstrates the well known fact that this acid is not ordinarily synthesized in the rat, again indicating that the increased liver triglycerides must have been formed from fatty acids which had been mobilized from adipose tissue.

THE PITUITARY

The pituitary has an important role in causing the rise in liver triglycerides, for these were not elevated by alcohol administered to hypophysectomized rats (Table III). These results are in accord with those of Mallov and Bloch[3] who reported that the admin-

TABLE III

Inability of Alcohol to Elevate Liver Triglyceride Levels in Hypophysectomized Rats

Treatment	Liver Triglycerides	
	Intact Rats (mg./gm. ± S.E.)	Hypophysectomized Rats (mg./gm. ± S.E.)
Saline (8 ml./kg. orally).....	5.3 ± 1.2 (3)	3.6 ± 0.6 (3)
Alcohol (3.4 gm./kg.)..	21.6 ± 2.2 (6)	4.4 ± 0.9 (6)

NOTE: Alcohol, in a 50 per cent solution by volume, was given orally to intact and hypophysectomized female Sprague-Dawley rats weighing 175 to 200 gm. (obtained from Hormone Assay Laboratories, Chicago); they were killed eleven hours later. Figures in parentheses represent number of animals.

TABLE IV

Prevention of Alcohol-Induced Triglyceride Deposition in Rat Liver by Adrenergic Blocking Agents

Procedure*	Liver Triglycerides (mg./gm. ± S.E.)
Saline (12 ml./kg. orally).....	4.8 ± 0.23 (4)
Alcohol, 4.8 gm./kg.....	12.8 ± 0.30 (5)
Dibenamine and alcohol.....	4.0 ± 0.06 (4)
Phenoxybenzamine and alcohol.....	5.1 ± 0.14 (5)
Ergotamine and alcohol.....	4.4 ± 0.05 (6)

NOTE: Alcohol, in a 50 per cent solution by volume, was given to NIH female Sprague-Dawley rats weighing 150 to 200 gm. that were killed eighteen hours later. Figures in parentheses represent number of animals.

* Dibenamine hydrochloride (50 mg./kg.) was injected subcutaneously twenty-four and forty-eight hours before the alcohol was given. Phenoxybenzamine hydrochloride (10 mg./kg.) was injected subcutaneously twenty-four hours before the alcohol was given. Ergotamine tartrate (2.5 mg./kg.) was injected subcutaneously immediately before the alcohol was given.

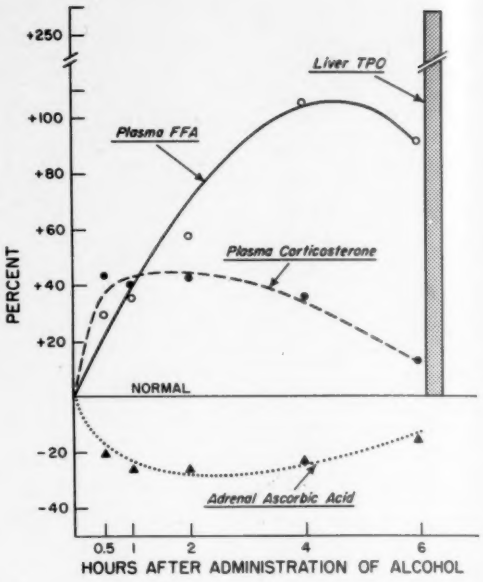

FIG. 1. Changes in plasma corticosterone, plasma free fatty acids (FFA), adrenal ascorbic acid, and liver tryptophane peroxidase after oral administration of alcohol, 4.8 gm./kg. as a 50 per cent solution, v./v. Each point is the mean of values obtained from twelve to fifteen rats of either sex. No sex difference was observed. Sprague-Dawley rats weighing 160 to 200 gm. were used.

istration of alcohol did not cause a rise in total lipids in hypophysectomized rats. This suggested that the triglyceride deposition was mediated through hormones released from the pituitary. We determined, therefore, the effect of alcohol on the pituitary-adrenal axis. It was found that alcohol depleted adrenal ascorbic acid, elevated the level of plasma corticosterone, increased the activity of liver tryptophane peroxidase, and elevated the level of plasma free fatty acids (FFA) (Fig. 1). Similar responses were elicited by exposure to certain stressful situations such as cold, intradermal formaldehyde and fear. The rise in FFA, incidentally, is another strong indication that the liver triglyceride deposition after alcohol is causally related to the mobilization of fatty acids from adipose tissue.

The administration of alcohol increased liver triglycerides to a much greater extent in female rats than in male rats. In preliminary studies, alcohol induced about the same pituitary responses in both sexes as shown by loss of adrenal ascorbic acid, rise in liver tryptophane peroxidase activity, and rises in plasma corticosterone and FFA. Now under study is the possibility that the difference between the sexes may concern liver enzymes that form triglycerides from fatty acids and glycerol.

The mechanism by which alcohol mobilizes fat is still unknown. Alcohol obviously causes the pituitary to liberate ACTH and perhaps other hormones important in mobilizing fat, but other factors are also involved. For example, pretreatment of rats with the adrenergic blocking agents, Dibenamine,® phenoxybenzamine (Dibenzyline®), or ergotamine, prevented the alcohol-induced deposition of fat in the liver (Table IV). The administration of Dibenamine also prevented the depletion of adrenal ascorbic acid and the rise in liver tryptophane peroxidase. Dibenamine may affect triglyceride deposition by blocking the action of norepinephrine at nerve endings which innervate fat cells or by preventing the release of pituitary hormones. These possibilities are under study.

The question arose whether the effect of alcohol on the pituitary was due to the pres-

ence of alcohol in the bloodstream or to a reflex action elicited by gastric irritation. Alcohol was therefore administered by slow intravenous infusion. Again a marked triglyceride deposition was observed, showing that alcohol does exert a systemic effect on the pituitary.

COMMENTS

A number of unsolved problems are raised by these experiments which show that single doses of alcohol can produce triglyceride deposition in liver through derangement of the fat transport. By what mechanism does alcohol cause the pituitary to release ACTH and perhaps other hormones? How do adrenergic blocking agents prevent the fatty deposition, and does this have clinical implications in the treatment or prevention of Laennec's cirrhosis? Is the acute response to alcohol related to the chronic response?

We suggest that the chronic and acute conditions have something in common. For one thing, the dose of alcohol that produces fatty deposition in rats is close to that taken by a human subject who poisons himself with six double martinis. Since alcohol is metabolized more slowly in man than in the rat, it is possible that daily consumption of a large amount of alcohol can cause "reversible" fatty deposition to become "irreversible." In addition, there is said to be a twofold range in the rates at which different subjects metabolize alcohol, so that the daily dose required to elicit triglyceride deposition in the liver might be considerably smaller in some persons than in others.

SUMMARY

Single large doses of alcohol in rats promote a pronounced rise in liver triglycerides which is maximal in fifteen to twenty hours and disappears in thirty to fifty hours. The proportion of linoleic acid in the triglyceride deposited in liver is virtually the same as in the triglycerides of adipose tissue. Since linoleic acid is not synthesized in the rat, the liver triglycerides must have been formed from fatty acids mobilized from adipose tissue.

The action of alcohol is apparently mediated through hormones released from the pituitary gland and can be explained in part through stimulation of the pituitary-adrenal axis. The deposition of triglycerides in liver is blocked by the administration of adrenergic blocking agents, suggesting that catecholamines are also involved in the mobilization of fat.

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Stimulation of Hepatic Fatty Acid Synthesis by Ethanol

CHARLES S. LIEBER, M.D.* AND RUDI SCHMID, M.D.†

FATTY infiltration of the liver is commonly found in alcoholic patients, but the relationship between excessive alcohol intake and fat accumulation in the liver is much debated.[1] Recently, studies were reported *in vitro*[2] suggesting that in addition to inducing nutritional deficiencies,[3,4] ethanol may have a direct metabolic effect on liver cells. The nature of this effect and particularly the relationship between ethanol and fat metabolism has been investigated in liver slices *in vitro*,[5] and the results obtained are summarized in the present article.

Liver slices of seventeen male rats taken at random were incubated *in vitro* for three hours with either 10 mM. ethanol or 10 mM. acetate. At the end of the incubation period, the total fatty acid concentration averaged 0.885 mg. palmitic acid per milligram tissue nitrogen on incubation with ethanol, and 0.805 mg. palmitic acid on incubation with acetate. In these seventeen paired determinations, the mean difference was 0.080 ± 0.029 mg. palmitic acid per milligram tissue nitrogen ($p < 0.02$). In nine instances, slices were incubated with either 10 mM. ethanol or 5 mM. glucose. At the end of the incubation period, the total fatty acid content of the slices averaged 0.901 mg. palmitic acid per milligram of tissue nitrogen on incubation with ethanol, and 0.831 mg. palmitic acid with glucose. The mean difference was 0.070 ± 0.021 mg. palmitic acid per milligram of tissue nitrogen ($p < 0.02$).

In the following experiments, an attempt was made to elucidate the mechanisms responsible for this effect of ethanol *in vitro*. Using isotopically labeled compounds, it was found that at a low substrate concentration (0.5 mM.), incorporation of ethanol-C^{14} and acetate-C^{14} into hepatic fatty acids was similar, indicating that both ethanol and acetate serve equally well as fatty acid precursors. With a substrate concentration twenty times higher, however, labeling of the fatty acids was three to twelve times higher with ethanol-C^{14} than with acetate-C^{14}, suggesting that the metabolism of ethanol in the liver may have a stimulatory effect on the incorporation of 2-carbon fragments into fatty acids. This was confirmed by the results shown in Figure 1 which indicate that unlabeled ethanol markedly stimulated incorporation of a trace amount of acetate-C^{14} into fatty acids, as compared to unlabeled glucose or acetate. In adipose tissue, on the other hand, where lack of alcohol dehydrogenase activity prevents significant oxidation of ethanol, incorporation of labeled acetate into fatty acids was not enhanced on incubation with ethanol, suggesting that the observed stimulatory effect in liver tissue is dependent on ethanol oxidation.

Oxidation of ethanol in the liver is catalyzed by alcohol dehydrogenase,[6-8] with coupled reduction of diphosphopyridine nucleotide (DPN) to DPNH (Fig. 2). *In vitro*, perfusion

From the Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

* Research Associate, Thorndike Memorial Laboratory, and Instructor in Medicine, Harvard Medical School; † Assistant Physician, Thorndike Memorial Laboratory, Boston City Hospital, and Assistant Professor of Medicine, Harvard Medical School.

Presented in part at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

This study was supported by research grant A-1833 (C) U. S. Public Health Service, The Nutrition Foundation, Inc., and the U. S. Army Medical Research and Development Command, Department of the Army, under Contract No. DA-49-193-MD-0213.

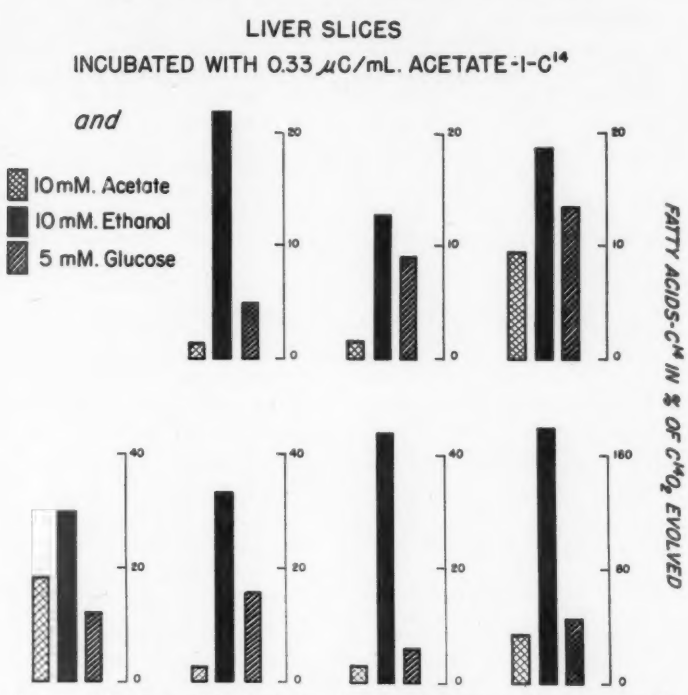

FIG. 1. Effect of unlabled acetate, ethanol and glucose on the incorporation of acetate-C^{14} into fatty acids in liver slices. From LIEBER, C. S. and SCHMID, R. *J. Clin. Invest*, 40: 394, 1961.[5]

Ethanol + $DPN^+ \rightleftharpoons$ Acetaldehyde + DPNH + H^+
Sorbitol + $DPN^+ \rightleftharpoons$ Fructose + DPNH + H^+

FIG. 2. Oxidation of ethanol and sorbitol in the liver.

of liver with ethanol results in reduction of the DPN : DPNH ratio,[9] and *in vivo*, administration of ethanol produces a decrease in DPN and an increase in DPNH concentration in the liver.[10] Furthermore, addition of exogenous DPNH to cell free liver extracts was found to stimulate fatty acid synthesis.[11,12] These findings suggest that the stimulatory effect of ethanol on the incorporation of acetate-C^{14} into fatty acids may be due to the excess DPNH formed on ethanol oxidation.

It has not yet been determined whether DPNH acts mainly by direct stimulation of fatty acid synthesis or by reducing the activity of the tricarboxylic acid cycle (TCA), or both. Diminished TCA cycle activity is suggested by the finding that unlabeled ethanol reduced the conversion of acetate-C^{14} to $C^{14}O_2$, as compared to incubation with unlabeled acetate. Moreover, addition of DPNH to liver homogenate has been found to reduce oxidation of TCA intermediates.[13]

Supportive evidence that the observed effect of ethanol on hepatic fatty acid metabolism may be due to a shift in DPN : DPNH ratio was obtained by incubating liver slices with another DPNH-generating system. In the liver, sorbitol is oxidized to fructose with concomitant reduction of DPN to DPNH[14] (Fig. 2). Like ethanol, incubation of liver slices with sorbitol resulted in increased incorporation of trace amounts of acetate-C^{14} into fatty acids. Conversely, a hydrogen acceptor such as methylene blue partly reduced the stimulatory effect of ethanol.

These observations suggest that in the liver the excess DPNH formed on ethanol oxidation results in a shift in the relative disposition of acetyl-CoA in such a way that more acetate is incorporated into fatty acids, while less is oxidized via the TCA pathway.

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Experimental Studies on the Role of Alcohol in the Pathogenesis of Cirrhosis

GERALD KLATSKIN, M.D.*

ALTHOUGH there is an impressive body of evidence implicating alcohol as an important etiologic factor in cirrhosis, the precise way in which alcohol affects the liver is still unknown. At the present time it is generally believed that the cirrhosis seen in alcoholics is a nutritional disorder related to the reduction in food consumption that often accompanies excessive drinking. According to this view, alcohol has no direct effect on the liver, but merely leads to a dietary deficiency of nutriments, and especially of lipotropic agents, essential for the maintenance of normal hepatic structure. The latter comprise a group of compounds including choline, a constituent of phospholipid, the amino acid methionine, which provides an exogenous source of labile methyl groups for the synthesis of choline, vitamin B_{12} and folic acid which, in some species at least, are essential for the *de novo* synthesis of labile methyl groups, and certain other lipotropic amino acids unrelated to choline, such as threonine, lysine and tryptophan. In experimental animals it is well known that a deficiency of these dietary factors leads to fatty infiltration and ultimate fibrosis of the liver.

The evidence usually cited in support of the dietary origin of alcoholic cirrhosis may be summarized as follows: (1) a high proportion of alcoholics in whom cirrhosis develops are malnourished, (2) dietary supplements of protein have proved to be of therapeutic benefit in such persons, (3) a similar type of cirrhosis is known to occur in nonalcoholics in parts of the world where the native diet is deficient in protein, and (4) there is a striking resemblance between the lesions of alcoholic cirrhosis and those seen in animals maintained on diets low in lipotropic activity.

ROLE OF ALCOHOL

Although these observations indicate that nutritional factors may be important in the pathogenesis of Laennec's cirrhosis, they do not necessarily imply that the effects of alcohol on the liver are solely due to limitation of the dietary intake. Indeed, there are a number of observations that contradict this view and suggest that alcohol may play a more direct role in the development of cirrhosis: (1) in some alcoholics cirrhosis develops despite an adequate dietary intake, (2) cirrhosis is rare in nonalcoholics with chronic wasting diseases accompanied by severe malnutrition, and (3) it has been demonstrated[1] that the mere withdrawal of alcohol in cirrhotic subjects maintained on a suboptimal diet often leads to marked improvement in the clinical, functional and histologic status of the liver, and that dietary supplements under such conditions are without additional benefit; in short-term studies[2] similar improvement has been observed in cirrhotic subjects receiving diets virtually devoid of protein.

Clinical observations such as these, while in no way discrediting the importance of nutritional factors in certain types of cirrhosis, do suggest that the effects of alcohol on the liver may not be attributable solely to changes in dietary intake, and have stimulated several

From the Yale University School of Medicine, New Haven, Connecticut.

* Professor of Medicine.

The studies reported were supported by a research grant (A-714) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

Presented at National Vitamin Foundation Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of the National Vitamin Foundation, Inc., March 1, 1960, New York, New York.

groups to investigate this problem in experimental animals.

In 1947 Ashworth[3] reported that in rats maintained on a high protein diet and given large amounts of alcohol for a period of fifty days grossly fatty livers developed. Since this was not observed in pair-fed control animals receiving identical amounts of protein but no alcohol, Ashworth concluded that the accumulation of fat was a direct effect of alcohol on the liver unrelated to dietary factors. This interpretation is open to question since (1) the large amount of alcohol used produced periods of coma resulting in a poor dietary intake and weight loss, and (2) no account was taken of alcohol calories, so that the animals receiving alcohol had a higher caloric intake and lost less weight than their pair-fed controls. Accordingly, the occurrence of fatty infiltration of the liver in the alcoholics could have been due to their greater caloric intake, or, conversely, the failure of fatty liver to develop in the controls could have been related to their greater weight loss.

Best and his associates[4] carried out similar studies, incorporating a number of important modifications designed to obviate the deficiencies in the Ashworth experiment: (1) alcohol was administered as a 15 per cent solution in lieu of drinking water, a technic that permitted a good dietary intake and satisfactory growth, (2) as controls, both pair-fed and isocaloric pair-fed animals were investigated (the latter group received an isocaloric supplement of sucrose in lieu of alcohol), (3) the basal diet was kept marginal with respect to lipotropic activity to facilitate detection of alcohol-induced changes in the nutritional status of the animals, and (4) parallel groups of alcoholic and control animals were given supplements of casein, choline or methionine.

When the animals were sacrificed at the end of six months, it was found that both the alcohol-fed animals and their pair-fed isocaloric controls (receiving a sucrose supplement but no alcohol) exhibited fatty infiltration and fibrosis of the liver. In both groups supplements of casein, choline or methionine prevented these hepatic changes. No hepatic lesions were found in the pair-fed control animals whose caloric intake was lower. Since the effects of alcohol and an isocaloric equivalent of sucrose appeared to be identical, and since they could be prevented in either case by supplementing the basal diet with lipotropic substances, Best concluded that alcohol had induced a choline deficiency by augmenting the caloric intake.

Experiments carried out in our own laboratory[5] confirmed Best's observation that alcohol and isocaloric sucrose supplements induce fatty infiltration and fibrosis of the liver in rats maintained on a diet of marginal lipotropic activity. However, it was found that reducing the caloric intake at the expense of sucrose in the basal diet enhanced rather than abolished the effects of alcohol on the liver. This suggested (1) that if alcohol increased the choline requirement it did not do so by augmenting the caloric intake, and (2) that the effects on the liver of alcohol and sucrose supplements were probably different despite the similarity of the hepatic lesions they produced. Although, as in Best's experiment, the protective effects of lipotropic substances suggested that alcohol had induced a relative choline deficiency, the possibility could not be excluded that alcohol had exerted a toxic effect on the liver leading to fatty infiltration and fibrosis, and that choline and methionine had merely hastened the removal of fat and thus prevented the subsequent development of fibrosis.

CHOLINE DEFICIENCY

In subsequent experiments[6] advantage was taken of the fact that in weanling rats choline deficiency produces a highly characteristic type of renal necrosis. Using such animals and the feeding technics previously described, it was shown that in alcohol-fed animals a significantly higher incidence of renal necrosis developed than in their pair-fed isocaloric controls, and that this effect of alcohol could be prevented by supplementing the basal diet with 0.08 per cent choline chloride. These findings were interpreted as confirmatory evidence that alcohol increases the choline requirement, and thus induces a state of

relative deficiency when the diet is marginal in lipotropic activity, and that this effect is not dependent upon augmentation of the caloric intake.

Our more recent studies[7] have been concerned with possible mechanisms involved in alcohol-induced choline deficiency. These have demonstrated that (1) triethylcholine protects against effects of alcohol, suggesting that alcohol induces a deficiency of choline itself rather than of labile methyl groups, (2) isocaloric equivalents of palmitic acid do not affect the choline requirement like alcohol, (3) the alcohol effect is not abolished by supplements of folic acid, vitamin B_{12} or the lipotropic amino acids, lysine, threonine or tryptophan, and (4) the deficiency created by alcohol is not dependent upon a defect in choline absorption, synthesis or utilization. Experiments in adult rats have revealed that under conditions of positive nitrogen balance alcohol spares nitrogen, but that under conditions of negative nitrogen balance alcohol increases nitrogen losses in the urine, principally in the form of urea. However, in neither situation does alcohol increase fecal nitrogen or lead to urinary losses of methionine or other amino acids, so that it is unlikely that alcohol-induced choline deficiency is related to changes in nitrogen balance.

Of particular interest has been our observation that in adult rats maintained on a 12 per cent casein diet and given a 15 per cent solution of alcohol in lieu of drinking water for a period of twelve days lactescence of the serum develops with a striking increase in serum triglycerides, changes not seen in pair-fed isocaloric control animals. Currently we are investigating the possibility that this hyperlipemia is the consequence of an alcohol-induced increase in fat mobilization from the depots. That such mobilization may occur is suggested by the report of Mallov and Bloch[8] that alcohol raises the fat content of the liver acutely and that this effect can be prevented by prior adrenalectomy or hypophysectomy, procedures known to suppress fat mobilization from the depots. If, indeed, alcohol does mobilize fat from the depots to the liver, and if, as Lieber[9] has suggested on the basis of recent *in vitro* studies, alcohol increases fat synthesis in the liver, it is conceivable that the apparent increase in the choline requirement induced by alcohol may be related to the acceleration of fat turnover in the liver. This possibility is currently under investigation.

Although the emphasis in this symposium has been on the nutritional and metabolic effects of alcohol on the liver, it should be pointed out that other environmental or constitutional factors may be of importance in the pathogenesis of Laennec's cirrhosis in the alcoholic. These have received little attention, but the fact that in only a small fraction of those who die of chronic alcoholism does cirrhosis develop[10] strongly suggests that overindulgence in alcohol and poor dietary habits may not be the only factors involved. Certainly the significance of genetic factors, intercurrent infection and exposure to other toxins in alcoholic beverages merit further investigation.

SUMMARY

The evidence available at the present time suggests that alcohol may induce a relative deficiency of choline leading to fatty infiltration and fibrosis of the liver by (1) limiting food consumption, (2) increasing the choline requirement, and (3) possibly by augmenting the caloric intake. In addition alcohol may raise the level of fat in the liver by stimulating fat synthesis and by mobilizing fat from the depots. Whether these latter actions are responsible for the apparent increase in the choline requirement induced by alcohol and whether they play a significant role in the development of cirrhosis is still uncertain. Although these nutritional and metabolic effects of alcohol appear to be important the possibility must be considered that other environmental or constitutional factors are involved in the pathogenesis of Laennec's cirrhosis in the chronic alcoholic.

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DISCUSSION OF PAPERS BY DRS. WESTERFELD; BRODIE, BUTLER, HORNING, MAICKEL, MALING; LIEBER, SCHMID; AND KLATSKIN

Dr. N. R. Di Luzio (*Knoxville, Tennessee*): Our interest in hepatic complications of ethanol began on reading a paper by Danopoulous (*Ztschr. ges. exper. med.*, 103: 212, 1938) which stated that ethanol was metabolized in the hepatic Kupffer cell. The alcohol tolerance curve was employed by Danopoulous et al. (*Acta med. scandinav.*, 148, 1954) to evaluate the functional activity of the reticuloendothelial system in man. I know of no evidence that confirms or denies the concept of Kupffer cell participation in ethanol metabolism.

In an attempt to evaluate this hypothesis, we began to study the effects of acute doses of ethanol and observed the fatty liver which was described by Mallov (*Am. J. Physiol.*, 184: 29, 1956). In view of Feigl's observations (*J. Biochem. Ztschr.*, 92: 282, 1918) on alterations in plasma lipid in acutely intoxicated persons, a condition he described as a "lipemia of intoxication," a study was also conducted on the concentration of plasma lipid in acutely intoxicated rats (*Am. J. Physiol.*, 194: 453, 1958). This study indicated that the concentration of plasma phospholipid, cholesterol and neutral fat was unaltered eight and sixteen hours after the administration of ethanol, at which times significant increases occurred in the concentration of liver triglyceride. The increase in liver neutral fat was not prevented by the simultaneous oral administration of choline chloride, nor was it associated with alterations in liver or plasma phospholipid metabolism. Subsequent studies with the continuous intravenous infusion of choline chloride, which was started immediately after the oral administration of ethanol, confirmed the failure of choline to prevent the development of fatty liver. These studies indicate that it is unlikely that the fatty liver induced by the acute ingestion of ethanol is due to the production of a choline-deficient state.

An attempt has also been made in ethanol-treated rats to relate the accumulation of lipids in the liver to increased mobilization of peripheral fat. The determination of plasma free fatty acids in fasted rats given either saline, glucose or ethanol demonstrated no significant difference between the ethanol- and the isocaloric glucose-treated rats. Since mobilization of peripheral fat can be blocked by the administration of glucose, the ethanol-treated rats were given an infusion of glucose but there was no alteration in the development of fatty liver. These results seem to indicate that we are at odds with Dr. Brodie and his group.

I would like to ask Dr. Brodie whether or not Dibenamine® can reverse the fatty liver, i.e., is there a direct hepatic action of Dibenamine? This, of course, raises the question as to whether or not we are dealing with a lipotropic agent.

In reference to Dr. Lieber's report, I have conducted studies on hepatic fat oxidation as indicated by the rate of deiodination of an I^{131}-labeled triolein emulsion in acute ethanol-treated rats. Our results are in agreement with what he has reported.

In reference to Dr. Klatskin's report, there is no question that the etiology of the acute ethanol-induced fatty liver is really quite different from the fatty liver induced by the chronic administration of small amounts of ethanol. The latter is responsive to choline and the former refractory to choline, at least in the doses and the mode of administration which we have employed.

Dr. Charles S. Davidson (*Boston, Massachusetts*): We will save the questions for the end of the period. Dr. Schmid?

Dr. Rudi Schmid (*Boston, Massachusetts*): We have heard two reports which dealt with the problem of the origin of the excess fat that is found in the liver of rats treated with alcohol. Dr. Brodie has presented findings which suggest that on alcohol administration fat is mobilized from the depots and is transported to the liver; Dr. Lieber has discussed data which he obtained *in vitro* suggesting that alcohol exerts a stimulatory effect on fatty acid synthesis in the liver. At first, these two observations may seem difficult to reconcile, but on closer inspection a possible explanation

to which Dr. Brodie has already alluded, becomes evident.

As Dr. Brodie has indicated, in his experiments the relatively large doses of alcohol used may have had a *pharmacologic* effect. Under these circumstances, alcohol may act like other hepatic toxins, such as chloroform and carbontetrachloride, which result in mobilization of fat from adipose tissue and its deposition in the liver. For reasons which are not yet clear, this transport process seems to be more active in female animals than in male animal; Dr. Brodie's studies were mainly carried out with female rats.

On the other hand, ethanol is not only a pharmacologic agent but also an excellent fuel which is largely metabolized in the liver. In Dr. Lieber's experiments, the incubation of liver slices was carried out with rather low ethanol concentrations. At this low concentration, alcohol probably has little pharmacologic effect, but serves primarily as a source of energy for tissue metabolism. Indeed, his findings suggest that the enhancement of hepatic fatty acid synthesis is dependent on oxidation of ethanol by alcohol dehydrogenase. It is this metabolic effect of alcohol which interests us, and which might provide a partial explanation for the accumulation of fat in the liver of rats in which alcohol has been substituted for part of the caloric intake.

With regard to the question by Dr. Di Luzio, it is apparent that at least in one of the proposed schemes for fatty acid synthesis both TPNH and DPNH are needed. Oxidation of ethanol may provide DPNH, while TPNH is derived from the hexose monophosphate pathway. On incubation of liver slices obtained from glycogen-depleted rats, ethanol was found to lack a stimulatory effect on fatty acid synthesis, probably because the reduced availability of TPNH was rate-limiting.

Dr. Davidson: Thank you for those stimulating comments, Dr. Schmid. Dr. György would you like to end the discussion? Dr. György needs no introduction; he is an old friend of mine and of most of us here. He has been working in fatty liver and many other fields for many years.

Dr. Paul György (*Philadelphia, Pennsylvania*): Three groups of what we may call older research workers—Dr. Sebrell, Dr. Daft and their colleagues, then Dr. Best and his colleagues, and myself and my colleagues—naively considered alcoholic cirrhosis and alcoholic fatty liver as belonging in the same category as many other deficiency diseases, such as alcoholic pellegra and alcoholic beriberi. We called it a nutritional disease.

Two years ago, at the meeting of the committee on metabolism (a subcommittee on liver diseases of the U. S. Army), our present chairman did not agree with those of us who called alcoholic cirrhosis a deficiency disease.

I am very glad we are not ostracized today, and that we are not ashamed to discuss alcoholic fatty liver and cirrhosis as a nutritional disease; even if it is assumed that alcohol produces, due to its metabolism in the liver, increased accumulation of fat, this still must be considered as a nutritional disease.

I am, of course, a little disturbed by Dr. Brodie's comments because I really do not know why I should protect myself against the effect of alcohol through diet and nutrition if it could only have a pharmacologic effect, as Dr. Davidson has just mentioned.

One objection has been raised to our naive basis, namely, that the fatty liver in alcoholics, or the liver in alcoholics, is different from the experimental fatty liver. It is interesting that nobody pointed this out.

It has been stated that neither Mallory bodies nor focal necroses is found in experimental fatty livers in rats.

I agree that Mallory bodies are not found in dietary fatty liver in rats with cirrhosis but, commenting on Dr. Schmid's last remarks, as to focal necrosis, cellular infiltration, as a sign of sequence of necrosis, is seen in rats in a very early stage of choline deficiency cirrhosis; as a matter of fact, within fifty days after rats are put on a choline deficient diet; this has been shown by Dr. Bras and myself a year or two ago.

We considered this the first sign of real liver damage, leading to fibrosis, and the fatty infiltration more or less only accidental.

I would like to make two more remarks: Commenting on Dr. Klatskin's objection to considering malnutrition as always being a cause of cirrhosis, even in alcoholics. He mentioned that in Japanese war prisoners, in concentration camps or in starvation states, cirrhosis does not occur.

It does not occur experimentally either, as has been first pointed out by Best. We have to have a sufficient caloric intake. If we do not have enough of a caloric intake, and the organism has to break down protein in the muscle and in other tissues, then the liver will be protected.

The final comments are of a more practical nature. We are speaking of alcoholic cirrhosis, and I heard only about the effect of pure ethanol. I defy anyone who would claim that so-called alcoholic cirrhosis is related to chemically pure ethanol.

We know that the people in Boston get cirrhosis from whiskey. In Australia they get it from beer, and in France from wine.

Those who read the interesting sequence of communications in The New Yorker on alcohol some months ago will remember that, in addition to alcohol, the alcoholic beverages—whiskey, beer and wine—have conjugers, aldehydes and other similar substances, whereas vodka is almost pure alcohol. It would be interesting to find out what the incidence of cirrhosis is in Russia.

The aromatic substances are of special interest to me because in France, in the perfume area, cirrhosis is extremely common, almost epidemic. There we may be dealing with "toxic" volatile substances which certainly should be considered.

In general, I was pleased to note from this present discussion that almost everybody at present considers alcoholic cirrhosis a nutritional disease. I still have an

open mind, and the effect of real toxic components in alcoholic beverages should not be discounted.

DR. DAVIDSON: Your comment on whiskey, beer and wine all producing or at least having something to do with cirrhosis in three different countries in the world reminds me of the book "Science, the Sacred Cow," in which there is an illustration of three people who are obviously tipsy; in front of one is scotch and soda, in front of the second is bourbon and soda, and in front of the third is gin and soda. The common denominator, of course, is soda, and obviously that is the cause of the drunkenness.

So here we believe that alcohol must be the cause of the trouble because it is the least common denominator; maybe the pendulum will swing again and we can go elsewhere.

Now we will allow each of the speakers to answer the questions which have been put to them, or to conclude their remarks in view of what has been said by others.

DR. W. W. WESTERFELD (*Syracuse, New York*): There are two questions that I would like to raise. One has already been mentioned. The studies by Drs. Brodie, Di Luzio and Maloff, are essentially in agreement: if you give large doses of alcohol, you get a fairly rapid infiltration of the liver with fat which cannot be stopped by giving choline, but the doses of alcohol that have to be given in order to do this are relatively large. Some rats are almost into the comatose stage. I would like to know the lowest dosage in terms of grams per kilogram, or in terms of milligrams per cent blood alcohol, which will produce this kind of stimulatory effect and cause a transport of fat from the adipose tissues into the liver.

Another question I would like to direct to Dr. Lieber: Most of the studies that have been made have indicated that by the time the blood alcohol has decreased to zero levels, 90 per cent or more of the alcohol carbons can be accounted for as carbon dioxide. Therefore there is no gross storage of alcohol as fat. From your studies, it is not necessary for the carbons of the alcohol to be laid down as fat. It is possible that the hydrogens could be incorporated and the carbons could go off as carbon dioxide. Do you have any comments on this?

DR. DAVIDSON: Dr. Brodie?

DR. BERNARD B. BRODIE (*Bethesda, Maryland*): I think Dr. Di Luzio asked if an adrenergic blocking agent would hasten the removal of triglycerides from liver. We have not yet performed this experiment.

As to the question of whether there is a critical blood level of alcohol that causes stimulation of the pituitary, this seems to be so. Thus, there is no effect at 200 mg. per cent, a very slight effect at 300 mg. per cent, and a very marked effect at levels above 400 mg. per cent.

We should remind ourselves, however, that while these facts are true for rats of a particular inbred species, the critical level is not known in other animal species. For man, a rather heterogeneous species, the critical level might be quite variable. In other words, much lower doses of ethanol might affect the pituitary in some people than in others; incidentally, the literature contains many hints that people who drink abnormal amounts of alcohol have abnormal adrenals.

I have been told that in Italy, where the people are predominantly wine drinkers, there is not much liver cirrhosis—suggesting perhaps that the level of alcohol in blood is important. Perhaps they do not have a high enough level of alcohol to fire off the pituitary.

I would like to ask Dr. Lieber a question: He left us dangling when he said he had evidence that alcohol stimulated the synthesis of fatty acids in the liver *in vivo*. I think we would all like to know something about this.

DR. DAVIDSON: Dr. Lieber, there are several questions now posed to you.

DR. CHARLES S. LIEBER (*Boston, Massachusetts*): I will do my best. First, I should like to stress the sex differences of the animals used by various investigators. Dr. Brodie has used mainly female animals whereas we studied male rats exclusively. It is well known that the female animals are more susceptible to various agents which produce a fatty liver by fat mobilization, for instance, ACTH. Therefore, I should like to ask Dr. Brodie: Did you study male rats and what kind of results did you get with them in your experimental conditions?

The difference in the dosages of ethanol must also be stressed. We have used *in vitro* a concentration of 46 mg. per cent ethanol, which is ten times less than the blood levels of Dr. Brodie's rats.

Concerning Dr. Westerfeld's question, I agree with him that one of the advantages of the presented explanation for the effect of ethanol consists in the fact that ethanol is able to stimulate fatty acid synthesis without having its carbon atoms necessarily incorporated into fatty acids. As a matter of fact, we have shown that ethanol *per se* is not a better precursor than acetate for fatty acid synthesis, but oxidation of ethanol in the liver is accompanied by a decrease in the DPH: DPNH ratio. This biochemical change induces an increase in fatty acid synthesis irrespective of the fact that most of the carbon atoms of ethanol are lost to carbon dioxide.

Unfortunately there is no time left to answer Dr. Brodie's question in detail. But the *in vivo* experiments indicating stimulation of hepatic fatty acid synthesis by ethanol will be published in the near future.

DR. GERALD KLATSKIN (*New Haven, Connecticut*): With respect to Dr. Brodie's comments regarding the nonspecificity of the reaction of fat mobilization induced by ethanol, it is noteworthy that Mallov has demonstrated that propanol also increases liver fat, although the metabolism of propanol is very different from that of ethanol.

Mallov believes that the mobilization of fat induced by ethanol is mediated through the hypophyseal-adrenal axis, and in rat experiments lasting two months demonstrated an increase in adrenal weight under the influence of ethanol. In our own long-term experiments lasting seven months, the administration of alcohol has

not led to an increase in adrenal weight. Admittedly, however, the late effects of malnutrition and the development of cirrhosis may have obscured the earlier effect of alcohol on the adrenal.

Although Dr. Di Luzio's failure to prevent the increase in liver fat induced acutely by the administration of ethanol suggests that the ethanol effect is not due to a deficiency of choline, it does not follow that under conditions of prolonged administration of alcohol choline will not prevent the development of hepatic lesions. Indeed, in our own long-term experiments supplementing the diet with choline prevented the development of the fatty infiltration and fibrosis which were found in pair-fed control animals receiving identical amounts of alcohol. This suggests that choline may facilitate the mobilization of fat from the liver and, thus, may prevent the subsequent development of fibrosis, even under conditions in which the induction of fatty liver is not due to choline deficiency, as in phosphorus poisoning.

On the basis of the evidence reviewed, it would appear that alcohol increases hepatic fat in at least three ways: (1) by increasing the mobilization of fat from the depots, (2) by increasing fat synthesis in the liver itself, and (3) by inducing a relative choline deficiency. It is quite possible that choline may prevent the accumulation of fat in the liver in all three situations by hastening its removal.

Clinical Reports

Nitrogen Balance as Affected by Neoplastic Disease and Its Therapy

DONALD M. WATKIN, M.D *

In the past six years studies have been conducted in which the over-all metabolism of nitrogen minerals, electrolytes and calories was measured in a group of sixty-eight subjects. Of this number, fifty-four had neoplastic disease, fourteen were either normal control subjects or persons with chronic illnesses of a non-neoplastic nature. A cursory grouping of these subjects permits an interesting observation. All normal subjects and all persons with chronic nonneoplastic disease (with the exception of three obese patients on 600 calorie diets) were in positive nitrogen balance. Of the fifty-four patients with neoplastic disease, seventeen were in positive balance, seventeen were in negative balance and twenty were in approximate nitrogen equilibrium.

This crude separation has two implications: (1) Normal subjects or chronically ill subjects fed an adequate diet in a metabolic study unit usually store nitrogen, (2) patients with neoplastic disease may respond normally by retaining nitrogen, may neither gain nor lose nitrogen or may lose nitrogen. In other words, patients with neoplastic diseases elected because they were well enough to be ambulatory, continent and cooperative on admission may display nitrogen metabolisms ranging all the way from positive to very negative nitrogen balance. How, then, can any sense be made out of the effects of neoplastic disease or of various therapeutic maneuvers on nitrogen metabolism?

The answer to this question lies in the fact that determining the histologic presence or absence of neoplasia (which traditionally has separated the patient with neoplastic disease from the patient without neoplastic disease) is a totally inadequate method gauging the effect of the tumor on the host or for that matter of estimating the prognosis of the host. Far more pertinent to host prognosis and host metabolism and to the topic of discussion is what may be called the *activity* of the neoplastic process. Activity is something which clinicians find easier to sense than to define; nonetheless, certain parameters of activity can be measured objectively and, together with good clinical judgment, permit division of patients with neoplastic disease into those with very active, moderately active and inactive neoplastic processes. Some of the parameters of activity are as follows: weight loss in the fact of an adequate dietary intake; an increase in the total number of calories expended per twenty-four hours (although not necessarily an increase in the basal metabolic rate); a low basal respiratory quotient when measured fourteen hours postprandially; a rapid rise in unesterified fatty acid concentrations with fasting; increased serum uric acid concentrations and increased twenty-four hour excretion of uric acid in the urine; rapid decrease in the thickness of skinfolds signifying

The studies reported were carried out at the National Institutes of Health, Washington, D. C. They were originally presented at the Scientific Session, Committee on Trauma, Division of Medical Sciences, National Research Council, Washington, D. C., June 10, 1960.

* Associate Professor of Nutrition, Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

a depletion of fat stores; increasing anorexia; and progressive anemia.

Except for two considerations negative nitrogen balance might be added to this list: (1) we are discussing nitrogen balance in neoplastic disease and, therefore, it would be totally unscientific to use the parameter under discussion as a means of dividing the patient material; (2) although patients with active disease may be in negative nitrogen balance, they are not always in negative balance and may, even in the most active stages, display positive nitrogen balances. Unfortunately, the total number of patients with neoplastic disease who have been investigated during controlled metabolic studies anywhere in the world is relatively small. Even this small number has not been systematically graded as to degree of neoplastic activity. Hence, it is impossible to present any valid statistics on the number of persons with active disease who display negative nitrogen balance, positive nitrogen balance or nitrogen equilibrium.

At this point, the question may justifiably be asked: why attempt to explain variations in nitrogen metabolism on the basis of disease activity when even in very active disease the entire range of nitrogen balances may be observed. The answer lies in the facts that (1) negative balances are not seen in the absence of active disease in patients on adequate protein, adequate calorie diets and (2) a net positive balance on the part of the host-tumor agglomerate does not mean that the host is storing nitrogen uniformly throughout his body. Hence, the answer to the question boils down to fact that division on the grounds of disease activity is only the first step in dispelling present ignorance of nitrogen metabolism not revealed by inspection of net nitrogen metabolism data from patients with active neoplastic disease.

To dispell some of this ignorance, we can first examine the data on net nitrogen metabolism, see what these data tell us about the untreated patients, and attempt to organize theories with regard to the internal metabolism of nitrogen which will, as a minimum requirement, not conflict with the available net nitrogen metabolism data. We can then observe changes in net nitrogen metabolism induced by certain physiologic and pharmacologic maneuvers and attempt to adapt our theory to the data from these investigations. We can, finally, with the help of new technics, delve more directly into the internal metabolism of nitrogen in the hope of acquiring data which will lend strong and more direct support to the previously developed theories. These more direct approaches must be made in conjunction with traditional studies of nitrogen metabolism and must be carried out during both step one and step two referred to herein. In addition, because of the interrelations of calorie and protein metabolism, the energy balance of the patients must be studied concurrently.

With this background, let us now look at some actual metabolic balance studies in patients both with and without active neoplastic disease.

MATERIALS AND METHODS

Four of the five patients whose studies will be discussed had chronic myelocytic leukemia (CML). The fifth had a lymphosarcoma. Each of the patients with CML participated in a control phase on a constant dietary intake prior to receiving a chemotherapeutic agent, either Colcemide®* or trimethyl colchicinic acid methyl ether d-tartrate (NCI 1136). The dietary intake was constant throughout the studies in patients with CML except for one (J. C. L.) whose intake was altered by frequent blood transfusions. The patient with lymphosarcoma participated in a study designed to test the effects of dietary calories and nitrogen, of the activity level of the disease and of radiotherapy on nitrogen pool size, metabolic pool turnover rate and the rate of incorporation of nitrogen into protein. Details of the design of this study are described subsequently.

During the metabolic investigations, the patients were hospitalized on a metabolic

* Colcemide used in this study was supplied through the courtesy of Dr. C. H. Sullivan, Ciba Pharmaceutical Products, Inc., Summit, New Jersey. Desacetylmethylcolchicine also appears in the literature as "Colcemid," "Demecolcine" and "Demecolcin."

study unit[1] and were under the constant supervision of specially trained nurses. A quantity of distilled water fixed for each patient was provided daily for drinking purposes. Patients were weighed daily in tared robes on kilogram scales accurate to 20 gm. immediately after the final voiding of the precisely timed twenty-four-hour urine collections. Blood specimens for chemical, liver function and certain hematologic studies were obtained from the antecubital veins after release of tourniquets in fasting patients. Blood specimens for differential white blood cell and platelet counts were obtained by finger puncture one hour after breakfast.

Voided urine specimens were pooled without preservatives in refrigerated glass jars until the closings of the twenty-four-hour collections. Ten per cent aliquots of these collections were layered into stoppered glass containers and stored in a freezer until pools of four accumulated. Prior to analyses, pools were thawed at room temperature and thoroughly mixed.

Individual stools were collected in tared stainless steel containers, weighed and pooled with other stools passed during each metabolic period. Carmine markers were used to identify the beginning and end of each stool collection period.

Refused food and emesis, if any, were saved, analyzed and subtracted from the daily intake for each period.

Food, feces and emesis were homogenized in a 5 L. Model CB-2 Waring Commercial Blendor. Weighed aliquots of homogenates were used for analyses of nitrogen and chloride. Other weighed aliquots were dried by lyophilization and pulverized and screened through a 40-mesh sieve in a Wiley mill. Weighed samples of the pulverized, dried material were ashed in Vycor crucibles at 400°c. prior to analyses for potassium, phosphorus, calcium and sodium.

Basal metabolic rate and respiratory quotient measurements were performed (when the patients were fourteen to fifteen hours postprandial following an overnight rest) by the open circuit technic using the Respirations Gasuhr des Max Planck Institüts for Arbeitsphysiologie, Dortmund.[2]

The following analytical methods were employed: urine, food and fecal nitrogen, macro-Kjeldahl;[3] potassium and sodium, internal standard flame photometer;[4] calcium, Kochakian and Fox;[5] phosphorus, Taussky and Shorr;[6] urine chloride, modified Sendroy;[7] food and fecal chloride, modification of the method described by Peters and Van Slyke;[7] uric acid, enzymatic method of Praetorius;[8] and oxygen and carbon dioxide, Scholander.[9]

The metabolic balance charts which follow are constructed according to the general scheme proposed by Reifenstein, Albright and Wells.[10] Intake is plotted downward from the zero line; fecal and urine outputs (or caloric expenditure estimated for each period by the insensible weight loss technic[11,12]) are then plotted upward from the line representing intake. When total output touches the zero line, equilibrium is represented; when output fails to reach the zero line, positive balance is represented; when output extends beyond the zero line, negative balance is represented. The ordinates of the N, K, P and Ca graphs are so selected that equal heights represent the approximate ratios at which these elements exist in normal protoplasm and bone, i.e., 3 gm. of N to 8 mEq. K to 200 mg. P (protoplasm) and 400 mg. Ca to 200 mg. P (bone). The ordinates of the Na and Cl graphs are so selected that equal heights represent the approximate ratios at which these elements exist in normal extracellular fluid, i.e., 150 mEq. Na to 100 mEq. Cl. Below the bar graphs, line graphs record the patients' weights. In addition to actual weights, theoretical weights based on considerations of the gain or loss of intracellular and extracellular fluid quantified by balances of N, K and Na are depicted and identified. The line graphs below the weight curves represent the serum uric acid concentrations and the bar graphs, the excretions of uric acid. The next lower bar graphs and dots indicate respectively the patients' basal metabolic rate and respiratory quotient. The line labeled "standard bar" indicates for each patient the age-predicted Mayo Foundation Normal Standard basal metabolic rate[13] and the respiratory quotient (0.82) of a normal subject on a standard mixed dietary.[14]

Other liver function, hematologic or blood chemical data appearing in the balance charts are self explanatory. The arabic numerals along the top and bottom of the charts refer to the metabolic balance periods of a particular study. Unless otherwise indicated, these periods were of four days' duration.

RESULTS AND COMMENTS

Figure 1 describes the study of a fifty-three year old man with active chronic myelocytic leukemia. For twenty days prior to therapy his average (periods 1 through 5) daily nitrogen balance was plus 0.79 gm. In these twenty days, he slowly gained a total of 0.3 kg. However, during these twenty days, his average daily caloric expenditure, estimated by measurement of insensible weight loss[11,12] was 789 calories greater than his dietary caloric intake, although his basal metabolic rate measurements were low or normal. Considerations of coordinate axis plots of the relations $N/P_{\alpha-\beta}$,* K/N and $K/P_{\alpha-\beta}$ suggest only that during these twenty days the patient was forming and destroying about equal amounts of tissue, whether normal or leukemic or both we cannot say; at any rate little if any leukemic tissue was being formed at the expense of the host. Sodium and chloride balances were slightly positive. Serum uric acid was increased to an average value of 11.6 mg per 100 ml. and the urinary excretion of uric acid averaged 975 mg. daily, indicating a high level of disease activity. The white blood cell counts were consistently in the range of 200,000 per cu. mm.

What theory can we devise which will, as a minimum requirement, be compatible with these data? The net nitrogen metabolism suggests equilibrium, since the amount of nitrogen retention is just large enough to balance the nitrogen losses occasioned by the growth of hair, nails, bones of hands and feet, cartilage of ears and nose and the desquamation of skin.[15] The slight gain in weight is readily accounted for by the apparent retention of 230 mEq. of sodium during the twenty days, an amount which must be reduced by unmeasured cutaneous losses.[16] The negative calorie balance implies an increased total daily energy expenditure compatible with increased energy requirements needed for tissue synthesis. The low or normal basal metabolic rate in the face of this increased total daily expenditure may indicate a diurnal cycle of energy expenditure perhaps related to a decreased work efficiency, an increased wastage of calories due to specific dynamic action or to increased tissue synthesis during periods of activity and food ingestion. Inspection of the $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ relationships on coordinate axis plots indicates that no large excesses of N, K or P are available for excretion as a result of internal shifts of nutrients within the host or between host and tumor. The increased serum uric acid concentration, the increased daily excretion of uric acid and the increased white blood cell count suggest that much leukemic tissue is being formed. However, the stability of the uric acid values and of the white blood cell counts and the insignificant deviations from normal in the coordinate axis plots of the $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ relationships suggest that the amount of leukemic tissue being formed is about equal to that being destroyed.

The total picture in this man, therefore, suggests the theory that the disease is active, but that, aside from demanding energy of the host, it is not making serious inroads into the host's protoplasm, at least not so long as he ingests 85 gm. protein and 2,500 calories daily.

Let us now take the second step and use a pharmacologic maneuver, the administration of the drug Colcemide (known to be effective in controlling chronic myelocytic anemia) to interrupt the theoretical relative homeostasis existing in this patient between tumor and host. In seven days, the patient received a total dose of 34 mg. of Colcemide. After a latent period of four days, dramatic changes occurred. In period 7, a daily negative nitrogen balance of 9 gm. was observed. The patient lost 1.4 kg. of body weight. His daily calorie deficit fell to 534. His basal metabolic rate was higher than control values and his respiratory quotient was that of a person burning pure fat.

* $P_{\alpha-\beta}$ = phosphorus balance corrected for the phosphorus associated with calcium in bone (Ca/2.23).

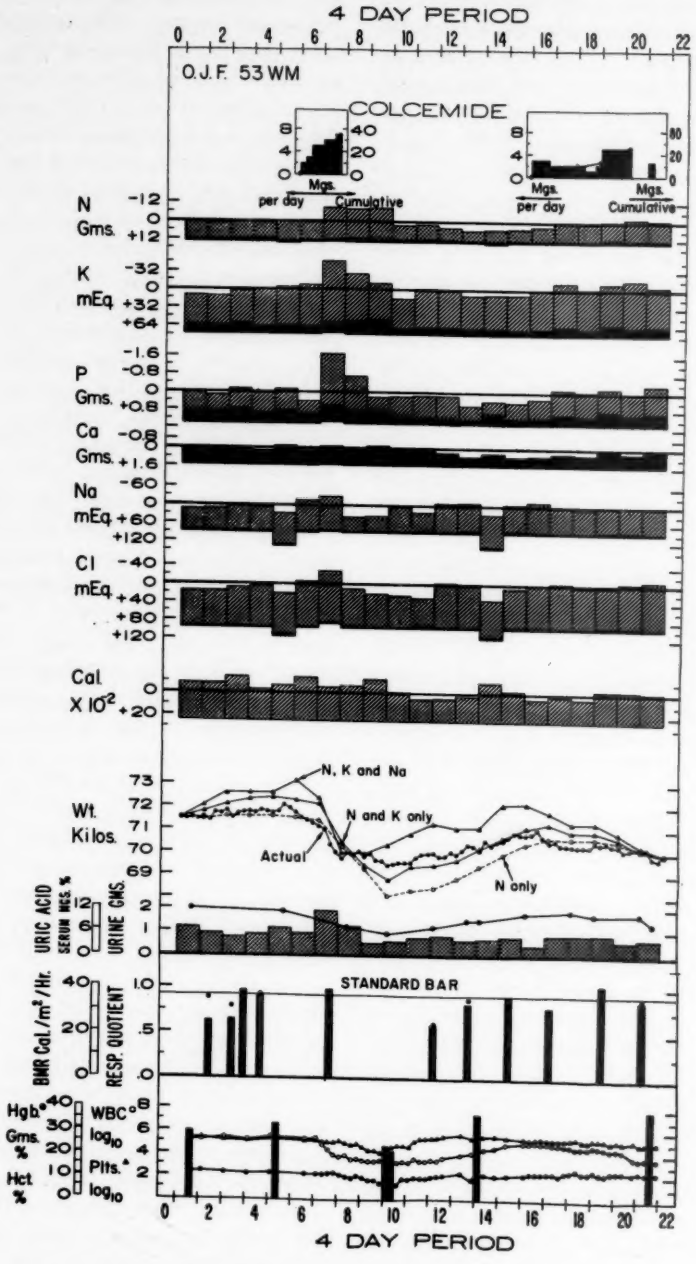

FIG. 1. A study of the metabolism of a fifty-three year old man with active chronic myelocytic leukemia before, during and after Colcemide therapy. See text for description and discussion. A similar figure appeared in *Fed. Proc.*, 18: 1155, 1959.

Inspection of the $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ relationships on coordinate axis plots suggests unequivocally that leukemic tissue is being destroyed and its components lost to the host-tumor agglomeration. Sodium and chloride balances were negative. The urinary excretion of uric acid rose to 1,900 mg. daily and concurrently serum uric acid concentration fell to approximately half of control values. The blood cell count fell precipitously to the range of 1,000 per cu. mm. Except for the balances of sodium, chloride and potassium and the excessive excretion of uric acid, essentially the same findings were observed over the course of the next two metabolic periods.

These findings support the following corollaries to our theory: The administration of Colcemide induced a total disruption of the formation of leukemic tissue. Existing leukemic tissue was destroyed resulting in excretion of N, K, P, Na and Cl and uric acid. Total energy expenditure fell, consistent with a decreased metabolic demand. Hence, with Colcemide, the disease has been arrested, the tumor has been destroyed and its components excreted and as before the host left, relatively speaking, alone.

The twenty-eight-day interval between the last day of period 9 and the end of the latent period following therapy was characterized by positive nitrogen balances, weight gain, positive calorie balances, relationships of $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ on coordinate axis plots suggestive of the formation of normal muscle protoplasm, equilibrium or negative balances of sodium and chloride, a lower but gradually rising serum uric acid concentration and urinary excretion of uric acid and a low but slowly rising white blood cell count.

These data support the theory that in the absence of an active leukemic process the host was able to retain nitrogen, to gain weight, to store calories, to retain N, K and P in such relationships as to indicate the formation of normal protoplasm and to retain little sodium or chloride. The gradual increases in serum uric acid concentrations and in the urinary excretion of uric acid together with the rise in white blood cell counts indicate a gradual return of activity of the leukemia.

It may be argued that the positive balances of N, K and P merely represent repletion of the nutrients lost during periods 7, 8 and 9. However, the coordinate axis plots have demonstrated that, while the constituents of leukemic tissue were lost, the constituents of normal muscle tissue are being retained. Hence, if repletion is occurring, it is repletion of the host and we must assume that our theory, in assuming that the leukemia had ignored the host except for feeding off his stores of calories, is in error. To rationalize this conflict between data and theory, we must assume that during our experiment the patient was eating enough in terms of protein and calories to protect his tissues from depletion by the tumor; in all probability, he had, prior to admission, eaten neither so much, so well nor so consistently. Interruption of the disease process inducing a marked reduction in total host-tumor agglomerate requirements permitted the host, when confronted with an identical dietary intake, to retain for his own repletion and maintenance nutrients formerly going to the tumor.

This detailed inspection of data from a single patient and, by deductive reasoning, the formation of a working hypothesis will enable us to review more rapidly a few additional studies in patients with chronic myelocytic leukemia showing other variations of nitrogen metabolism in active neoplastic disease.

The next chart (Fig. 2) describes the study of a fifty-four year old man who also had active chronic myelocytic leukemia. During the study he was on a diet constantly containing 90 gm. of protein and 2,600 calories. For twenty-eight days prior to therapy (periods 1 through 7) his average daily nitrogen balance was minus 1.7 gm. In these twenty-eight days, he slowly lost 0.2 kg. and his average daily caloric expenditure exceeded his dietary caloric intake by 666 calories. His average basal metabolic rate was above the standard for his age and his average respiratory quotient was below the standard. Coordinate axis plots of the relation $N/P_{\alpha-\beta}$, K/N and $K/P^{\alpha-\beta}$ suggest that the patient had a net loss of components of normal tissue. During these twenty-eight days, he had a net gain of 256 mEq. of sodium, to be reduced by an un-

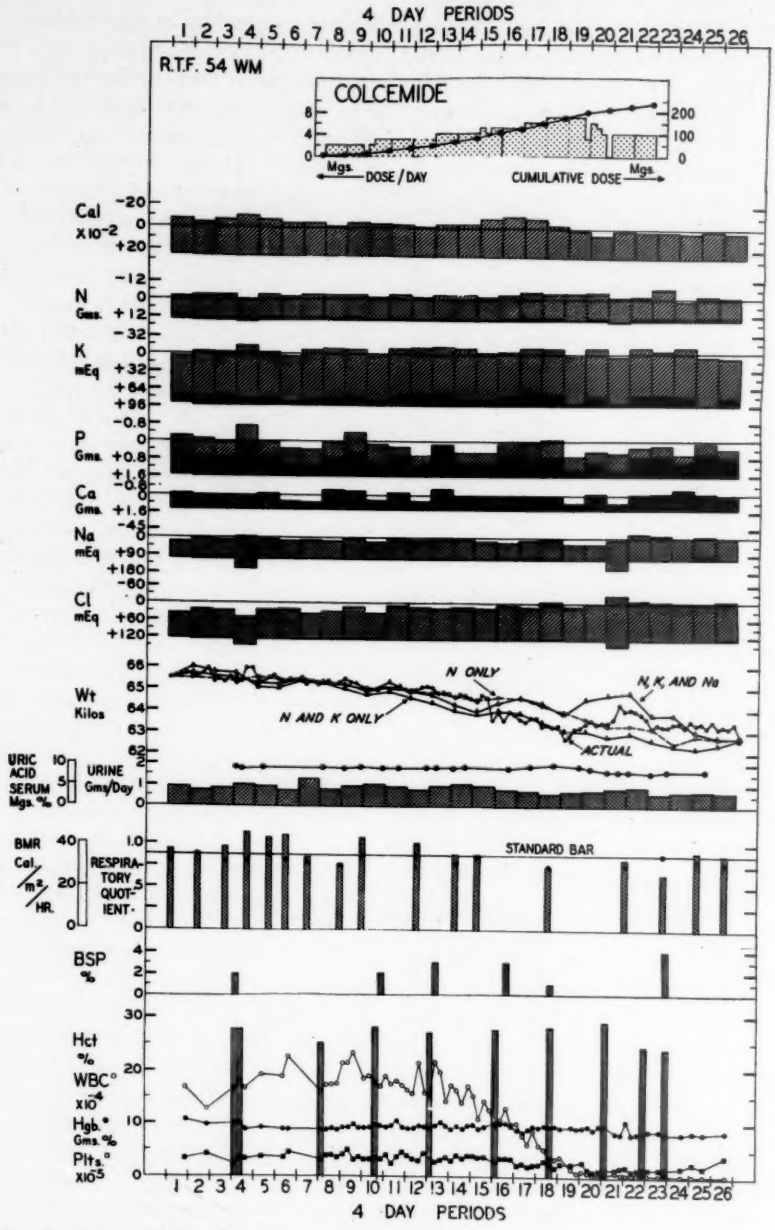

FIG. 2. A study of the metabolism of a fifty-four year old man with active chronic myelocytic leukemia before, during and after Colcemide therapy. See text for description and discussion.

measured cutaneous loss. He had an average serum uric acid concentration of 8.3 mg. per 100 ml. and an average daily uric acid excretion of 940 mg., indicating a high level of disease activity. The white blood cell counts were in the range of 175,000 per cu. mm. range and showed a slowly rising trend.

In this patient, Colcemide therapy was initiated slowly and the effective dosage range, as judged by a reduction in the white cell count, was not reached until period 13 at a level of 4 mg. Colcemide daily. In periods 13 through 20, nitrogen losses increased, body weight decreased and then increased, caloric expenditure first rose and then fell, the basal metabolic rates were considerably lower than control values, serum uric acid concentration rose slightly and urinary excretion of uric acid decreased. The white blood cell count fell to the range of 5,000 per cu. mm. In this patient, however, interpretation of the coordinate axis plots of the relations $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ is not easy since the ratios are negative, indicating that N is being lost while P is being retained. This is the situation anticipated in formation of leukemic tissue from the components of normal tissue. Why it should occur in the face of interruption of the leukemic process in this patient is not at all clear.

In periods 21 through 26, the patient received 4 mg. Colcemide daily for the first two periods and no Colcemide for the remaining four. During these twenty-four days, his average daily nitrogen loss was 2.16 gm. His weight varied but was the same at the end as at the beginning of the twenty-eight days. His total daily average energy expenditure, however, was 235 calories less than his dietary caloric intake. The basal metabolic rates were low or in the normal range. Inspection of balance ratios continued to reveal negative values. Serum uric acid concentrations fell but not to the normal range, and daily urinary excretion of uric acid fell but not to the normal range. The white blood cell count remained in the range of 5,000 per cu. mm.

This patient, even when in clinical remission and even when storing calories, was unable to retain nitrogen. The marked phosphorus retention, not apparently going to bone, remains unexplained. A possible clue to the mystery which we will explore later is revealed in the next chart (Fig. 3) which is that of the same patient studied for a second time during a hematologic relapse five months after completion of the first study. Note particularly the patient's initial weight, 70 kg., compared with a weight of 63 kg. at the end of the first study five months previously. In other words, while living at home on an *ad libitum* dietary intake, the patient had gained 7 kg., despite the negative N balance demonstrated just before his leaving the metabolic study unit. In passing, note the decreased caloric expenditure and white blood cell count with effective therapy as in the previous study. However, note also in periods 11 through 16 the increased nitrogen loss and losses of K, P and Ca with effective therapy. The average balance ratios are positive: $N/P_{\alpha-\beta}$, K/N and $K/P_{\alpha-\beta}$ and more indicative of the loss of normal tissue than of leukemic tissue. The gain in weight while at home may suggest merely a psychological reaction to the rigors of the metabolic study unit, as noted by Schottstaedt et al.[17] However, as will be discussed subsequently, other explanations may be involved[18–20] based on the observations that the patient may need a preliminary period comparable to the warm up of a gasoline engine as a prerequisite to nitrogen retention and weight gain. Premature termination of the first study may have prevented measurement of the anabolic process which occurred in this patient between admissions to the metabolic study unit.

The fourth chart (Fig. 4), that of a thirty-eight year old man, is presented mainly to show that even in the presence of active disease as evidenced by clinical symptoms, by an increase in serum uric acid concentrations, by an increase in the daily urinary excretion of uric acid and by a white blood cell count in the range of 400,000 per cu. mm. the host-tumor agglomerate prior to therapy was retaining 1.54 gm. nitrogen daily while at the same time displaying weight loss, an average daily energy expenditure of 1,042 calories greater than dietary intake and an elevated basal metabolic rate. This study certainly suggests retention of nitrogen by tumor except for the fact that

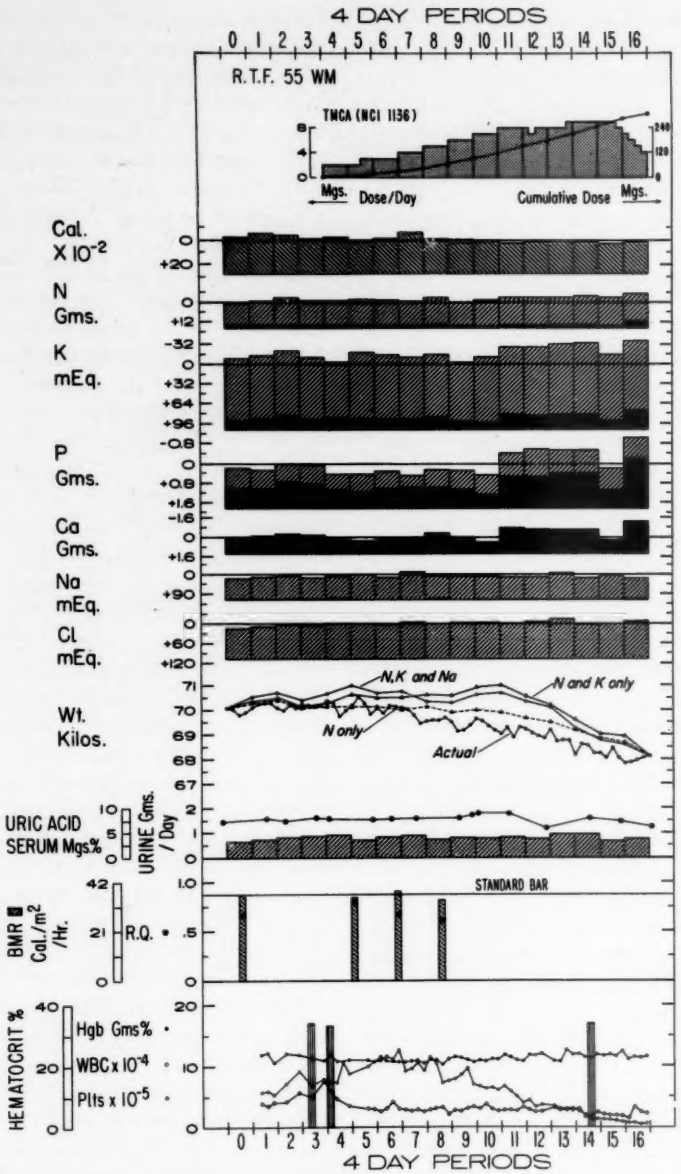

FIG. 3. A study in the same patient described in Figure 2 investigated a second time during an hematologic relapse five months after completion of his first study. Trimethyl colchicinic acid methyl ether d-tartrate (NCI 1136) was the therapeutic agent. See text for description and discussion.

the $N/P_{\alpha-\beta}$ balance ratio is negative and the white count actually fell to half its initial value during the control period.

The fifth chart (Fig. 5), that of a twenty-three year old woman previously treated, but refractory to Myleran,® is presented primarily to show the metabolic effects occasioned by relapse in the form of a so-called "blastic crisis."

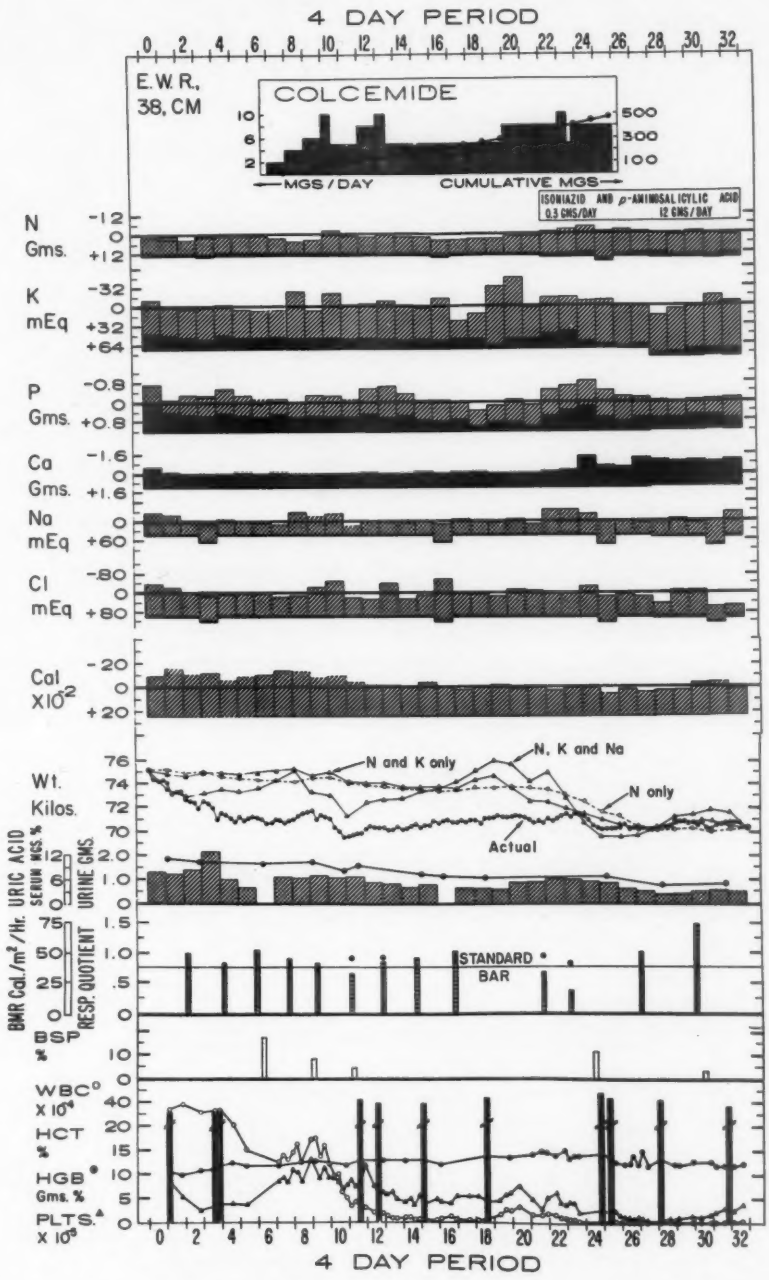

FIG. 4. A study of the metabolism of a thirty-eight year old man with active chronic myelocytic leukemia and pulmonary tuberculosis (diagnosed during the study) before, during and after therapy with Colcemide and before and during isoniazid and p-aminosalicylic acid therapy. See text for description and discussion.

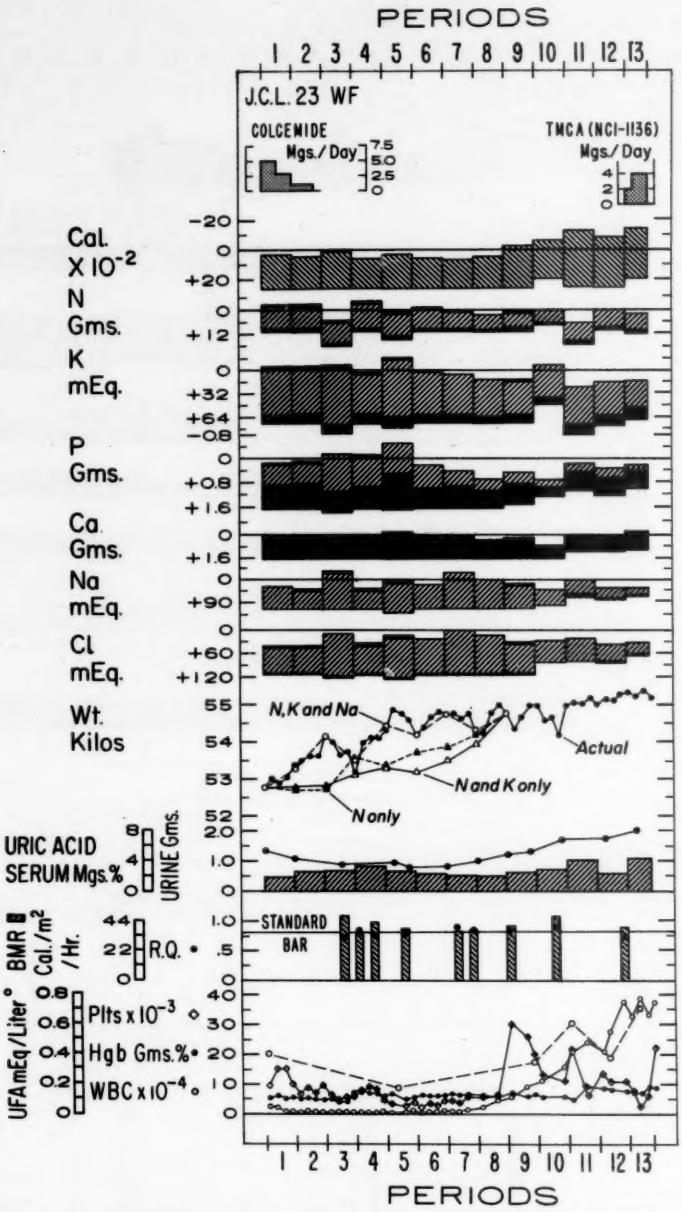

FIG. 5. A study of the metabolism of a twenty-three year old woman presented primarily to show the metabolic effects occasioned by relapse in the form of a so-called "blastic crisis." See text for description and discussion.

Note in periods 1 through 6 the average daily negative nitrogen balance of 2.05 gm. (disregarding blood transfusions), the gain in weight, the positive calorie balance of 431 calories daily, the negative phosphorus balance, the normal serum uric acid concentration, the normal daily urinary excretion of uric acid and the slightly elevated basal metabolic rate.

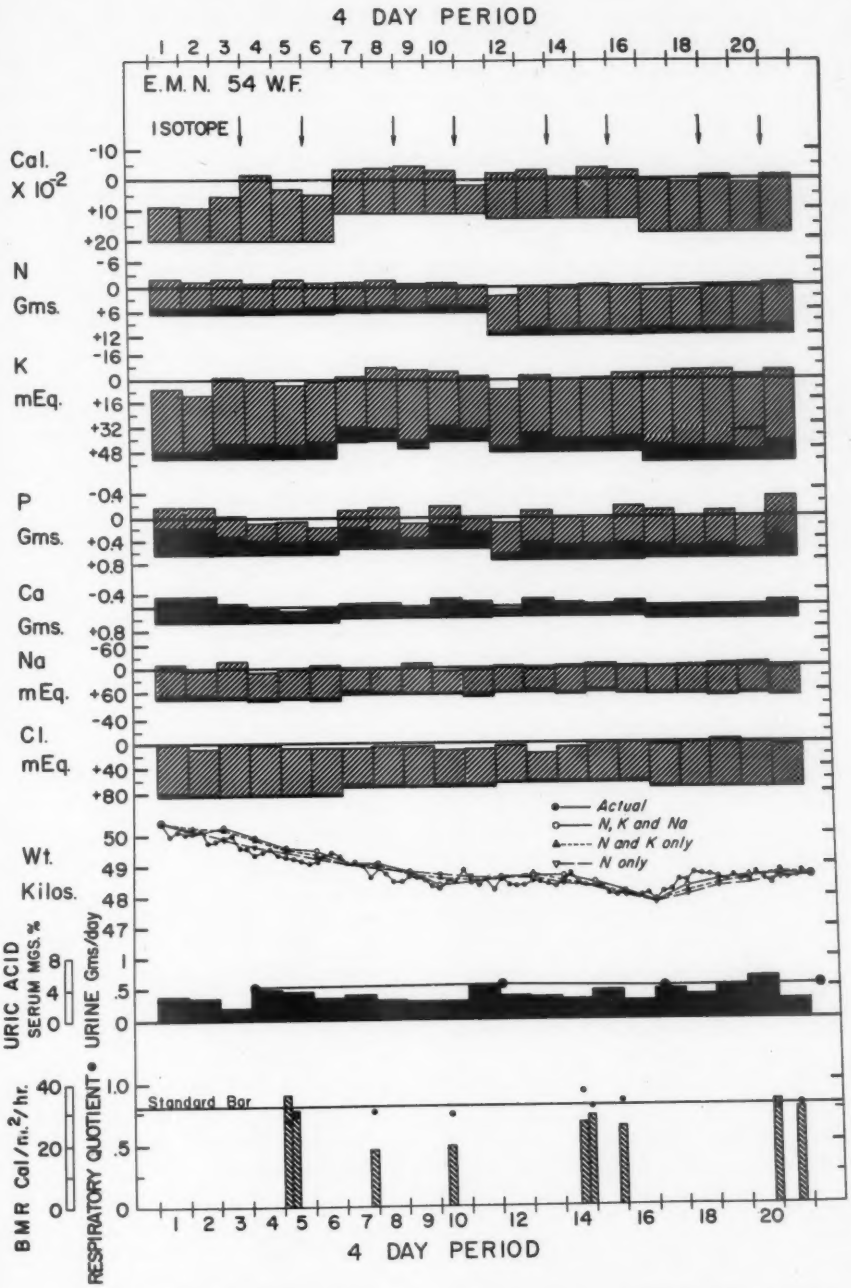

FIG. 6. A study of the metabolism of a fifty-four year old woman with lymphosarcoma during an inactive phase of her disease on four diets providing four combinations of calorie and protein intake. See text for description and discussion. A similar figure appeared in *J. Clin. Invest.*, 38: 892, 1959.

As the white blood cell count began to rise (period 7), several changes occurred: nitrogen balance became negative, potassium and phosphorus balances became positive, sodium and chloride retention was greater, the rate of weight gain was lower, serum uric acid concentrations increased and the daily urinary excretion of uric acid increased. In this patient, measurements of unesterified fatty acid,[21] after a fourteen-hour fast, were made before and during the blastic crisis; they are recorded as open circles connected by dashed lines at the bottom of the chart. Note the striking rise in the concentrations of unesterified fatty acid occurring simultaneously with a rise in the white blood cell count.

From this survey of a few metabolic studies of patients with chronic myelocytic leukemia, it is apparent that additional data are needed to enable us to explain convincingly the various metabolic patterns seen in various patients with active neoplastic disease. One such study has been completed on a metabolic study unit in collaboration with Tschudy and his colleagues.[18–20] In this study, in addition to the methods already described, the dynamic aspects of nitrogen were estimated by the San Pietro and Rittenberg model[22] for measurement of the nitrogenous metabolic pool size (P), the rate of nitrogen incorporation into the protein of the patient (S) and the rate constant for metabolic pool turnover (B). The study was designed to distinguish in quantitative terms between active and inactive neoplastic disease and to appraise the effects of varying the calorie and protein content of the diet. These studies were conducted in a fifty-four year old woman (weighing 50 kg.) with lymphosarcoma during an inactive phase of her disease while she was on four diets providing four combinations of calorie and protein intake and during an active phase on the low calorie, low protein diet used in the initial study, the metabolic chart of which is shown in Figure 6.

During the inactive phase of this patient's disease, a 40 gm. daily protein intake (periods 1 through 11) was associated with weight loss and negative nitrogen balance on both 1,800 calorie (periods 1 through 6) and 1,200 calorie (periods 7 through 11) diets. An 80 gm. daily protein intake (periods 12 through 21) was associated with slight nitrogen retention but continued weight loss on the 1,200 calorie diet (periods 12 through 16) and with slight nitrogen retention and weight gain on the 1,800 calorie diet (periods 17 through 21). The dynamic studies suggest that the rate constant for metabolic pool turnover (B) is relatively constant and unaffected by changes in calorie and protein intake over the range of dietary variation and period of time studied. The nitrogenous metabolic pool size (P) and turnover rate (BP) appear to increase somewhat when the dietary protein was increased from 40 to 80 gm. daily. The rate of nitrogen incorporation into the protein of the patient (S) increased only when both nitrogen and caloric intake were raised. Since, on the 80 gm. daily protein intake, the total nitrogen and urea excretion were essentially the same on both the 1,200 and 1,800 calorie diet, the greater incorporation into protein (S) corresponds to more rapid turnover of the metabolic pool (BP) associated with the higher caloric intake.

Although S increased significantly on the high protein, high calorie regimen, the patient retained an average of only 0.32 gm. nitrogen daily, not significantly different from the 0.35 gm. nitrogen daily retained on the high protein low calorie regimen. This suggests that high protein, high calorie diets may increase protein turnover and incorporation when there is no demonstrable net increase in the amount of protein in the body. The patient, on returning home, gained 7 kg. during the course of the next six months during which time her activity, appetite and *ad libitum* food intake were increased. A similar increase in weight was observed under similar circumstances in one of the patients with chronic myelocytic leukemia previously discussed herein. This phenomenon poses the question as to whether increased protein turnover may be a necessary prelude to positive nitrogen balance.

One year later this patient returned to the metabolic study unit with active neoplastic disease and was again studied, this time on the 1,200 calorie, 40 gm. protein diet (the maximum

oral intake she could tolerate). An increase in nitrogenous metabolic pool size (P) but no change in the rate constant for metabolic pool turnover (B) were found. The metabolic pool turnover rate (BP) and the rate of nitrogen incorporation into protein (S) were increased as they had been in the preceding study when both calorie and protein intake were at their highest levels.

The results of the studies performed when the disease was inactive are in general agreement with investigations in animals,[23] in normal human subjects[24] and in patients with chronic liver disease,[25] suggesting that incorporation of nitrogen into protein is greatest when both calorie and protein intakes are high. The results of the studies performed when the disease was active suggest that the turnover rate of the nitrogenous metabolic pool (BP) and the nitrogen incorporation into protein (S) were equal to those observed during high calorie, high protein feedings.

This raises the question as to whether the consumption of more dietary calories and protein during periods of increased disease activity will further increase protein turnover and incorporation and, if so, whether these increases result in (1) net nitrogen retention or (2) merely increase energy and protein metabolism.

This hurried review will emphasize the lack of present knowledge of the effects of one type of chronic illness on the metabolism of nitrogen. The fact that additional studies of nitrogen kinetics have not been performed is a tribute to the difficult technology and the large numbers of personnel required, not to mention the necessity of opportunism when the right situation arises in the right patient. The need for better, simpler technology and more carefully planned investigations can best be dramatized by the many unanswered questions raised by this discussion.

SUMMARY

Normal subjects and those ill with chronic non-neoplastic diseases stored nitrogen when fed an adequate diet in a metabolic study unit; however, patients with active neoplastic disease demonstrated nitrogen equilibrium, positive nitrogen balance or negative nitrogen balance when studied under identical conditions.

Examples of variations in nitrogen metabolism in patients with neoplastic disease are shown by studies in four patients with chronic myelocytic leukemia: (1) The first was in negative calorie balance but nitrogen equilibrium while eating a diet adequate in protein; later, following a course of chemotherapy, he showed increased nitrogen retention. (2) The second was unable to retain nitrogen while under study even following remission but gained weight rapidly after returning home. (3) The third demonstrated nitrogen retention despite weight loss and negative calorie balance. (4) The fourth displayed the metabolic changes occurring during relapse in the form of a "blastic crisis."

To distinguish in quantitative terms between active and inactive neoplastic disease and to appraise the effects of varying the caloric and protein contents of the diet, a patient with lymphosarcoma was studied by classic metabolic balance and by N^{15}-labeled L-aspartic acid kinetic methods. Only on the high calorie, high protein diet did her nitrogen incorporation into protein increase, but even then her net nitrogen retention was no greater than on the lower protein and calorie diets. Later, in a more active phase of her disease, the patient showed increased nitrogen incorporation into protein, even though the diet was low both in calories and in protein. These findings suggest (1) that increased protein turnover may be an essential prelude to positive nitrogen balance and (2) that high protein and calorie dietary intakes during an active phase of neoplastic disease when protein turnover is already high may merely increase energy and protein metabolism without inducing net nitrogen retention.

The need for better and simpler technology and for more studies of the details of human nitrogen metabolism is emphasized by the numerous gaps in present knowledge pointed out by these investigations.

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Fibrinolytic Activity of Whole Blood from South African Bantu and White Subjects

ALEXANDER R. P. WALKER, M.SC., PH.D.*

MORTALITY from coronary heart disease is believed to be almost unknown among rural South African Bantu; even among urban dwellers, deaths from the disease as proved at necropsy are extremely few.[1,2] Confirmation of this belief is being obtained from a collaborative clinical and biochemical study now in progress on Johannesburg Bantu pensioners over sixty years old (some approaching 100 years of age); in 340 subjects thus far examined, only one case of coronary heart disease, judged from clinical and electrocardiograph observations, has been detected.[3] In the same number of elderly white people in Western countries, evidence suggests that fifty or more cases would be expected.[4,5]

This favourable situation among the Bantu is far from explicable. Thus, while severe atherosclerosis of the aorta and, to much lesser extent, of coronary vessels is not common among them, unquestionably on occasion severe lesions do develop.[6–11] Hypertension, moreover, is common.[6,7,9,12,13] Since these conditions, severe atherosclerosis and hypertension, are present in a proportion of adult Bantu, it would seem plausible from current thought[14,15] to consider that decreased coagulability and increase fibrinolysin activity of blood may be among the salient factors that prevent the occurrence of acute thrombotic episodes in the coronary vessels of these people. Studies on Cape Town Bantu have revealed a deficiency of some factors in blood coagulation, and a more efficient function with respect to other coagulation mechanisms.[16–20] Accordingly, it is at least arguable "that the Bantu should have more thrombotic disease."[17] It may be noted that the same difference prevails between Australian whites and New Guinea primitives, the latter apparently evincing "greater blood coagulability."[21] Regarding fibrinolysis, however, there is evidence of superiority of activity in Bantu males compared with white males, both in whole blood[16,20] and in plasma.[22,23] In pursuit of endeavours at this centre to throw light on why the Bantu are virtually free from death from coronary heart disease, it seemed highly desirable to confirm these findings and, in addition, to determine mean values in groups of Bantu of both sexes, primitive and sophisticated, as well as among white control subjects; and to learn, moreover, of the trend of changes in fibrinolytic activity evoked by a fatty meal in comparison with a nonfatty one, and by exercise in comparison with inactivity.

SUBJECTS AND METHODS

Subjects. All subjects were in outward good health and pursuing their usual avocations. *Rural Bantu:* These included nineteen male and twenty-one female Mocambique Shangaans (Chichumbane region), thirty-nine male and fifty female Bechuanaland Tswana (Kanye region), and sixty male and sixty female Transvaal Pedi (Jane Furse Hospital region, Sekhukhuniland). From Johannesburg these centres, respectively, are about 500 miles East, 250 miles West, and 200 miles North East; they were chosen deliberately to provide widely separated diverse primitive population groups. The diets consumed in these three

From the Human Biochemistry Research Unit, South African Institute for Medical Research, and Council for Scientific and Industrial Research, Johannesburg, South Africa.

* Head, Human Biochemistry Unit.

This investigation was supported in part by research grant H-4244 from the National Heart Institute, U. S. Public Health Service, Bethesda, Maryland.

regions are not identical, but, while probably adequate in calories and possibly in gross protein, they are low in animal protein, fat and cholesterol, and high in carbohydrate and crude fibre. *Urban Bantu males:* These subjects (ninety-three) largely were workers at this Institute; they consumed a partially westernised diet. *Urban Bantu females:* These subjects (fifty-six) were drawn from a variety of sources; they also consumed a partially westernised diet. *Whites:* These subjects (forty-four males and fifty-five females) mainly were workers at this Institute, habituated to a diet common to middle-class white people.

Ages. Most Bantu, particularly rural dwellers, are ignorant of their correct age. Hence, the mean ages recorded are approximate.

Collection of Blood Samples. In regard to time of collection of samples, there are two alternatives. (1) At first sight it is desirable that in studies of this nature, blood samples be collected under basal conditions, i.e., after a long period of fasting. However, one's past experience in field work on the Bantu has demonstrated the utter hopelessness of attempting to get nonhospitalised subjects to present themselves for blood collection at a specified time in a completely breakfastless state. Hence, if basal conditions are to be aimed at, the investigation is prejudiced from the start. (2) There is, however, a second aspect to consider. Fearnley et al.[24] have shown that differences of 100 per cent and even more in blood fibrinolytic activity may occur in specimens taken from the same person very early in the morning and late in the afternoon. The wide extent of this diurnal rhythm has been confirmed by Buckell and Elliott[25] in plasma. Limited studies by the former workers have suggested that fibrinolytic activity is roughly steady from just before noon until late afternoon or early evening (subsequent studies at this centre substantially have confirmed this finding). The alternatives, therefore, are either to investigate subjects in early morning at basal state, in which case attempts to study the effect on fibrinolytic activity of a fatty meal or of exercise will be partially masked by the diurnal rhythm; or, to study subjects at the steady state period but under the disadvantage of nonfasting conditions. Bearing in mind that observations at basal state virtually are out of the question, at least for primitive rural Bantu, it was finally decided to collect samples at the beginning of the steady state period, i.e., from 11.30 to 12.30 A.M., four to six hours after breakfast, which in composition was the subjects usual repast.

Method of Assay. The method of Fearnley et al.[24] using whole blood was employed, mainly because it is far easier to undertake under field conditions compared with methods using plasma, but also because of certain criticisms that have been made about the reliability of values obtained on plasma by Billimoria et al.[26] All glassware was washed with acid and sterilized with heat immediately prior to use. Apparatus was used at or near 0°C., as were all reagents, which were freshly prepared for each run. Determinations were carried out in triplicate, sometimes quadriplicate. Briefly, 5 ml. or more venous blood was withdrawn without stasis, and immediately 0.2 ml. was added to 1.7 ml. phosphate buffer (pH. 7.25)* and just previously added 0.1 ml. thrombin solution (Parke Davis & Co., Thrombin® topical, 50 units per ml. in sterile normal saline) contained in tubes (9 by 1.5 cm.) surrounded by ice water in a beaker. After thorough mixing, tubes were refrigerated for a half to one hour, and then incubated at 37°C. in a water bath fitted with glass windows. Lysis time (taken to the nearest quarter hour) was that taken by clots from start of incubation to time of disappearance, tubes being inspected every ten to fifteen minutes. The recorded figure was the mean of the values for the tubes used for each determination. When individual tube values differed from the mean by more than 10 per cent (which happened in 10 per cent of cases), results were rejected, and tests repeated on fresh blood samples.

It will be understood that the shorter the

* The buffer mixture specified by Fearnly et al.[24] and Billimoria et al.[26] has a pH value of about 7.25, not 7.4 as stated.

lysis time, the greater is the fibrinolytic activity in blood and vice versa.

RESPONSE TO FAT-FREE AND TO FATTY MEALS

The purpose of investigating the effect of the fat-free meal merely is to establish a base line against which the effect of the fatty meal may be judged. In seeking to determine the effect of the latter on fibrinolytic activity, there are two alternatives: (1) A very large intake of fat may be desirable in order to exaggerate the inhibitory or other effect produced in activity levels. (2) Notwithstanding, there is much to be said for limiting intake to amounts which are physiologic, i.e., not excessively divorced from everyday intake of the particular type of fat partaken of at a single meal. In the Bantu, this amount necessarily will be low, certainly much lower than white people are accustomed to. A compromise must therefore be reached. Before discussing the amount, it should be remarked that the feeding of butter fat, at least in the first instance, is desirable, partly on account of its place in everyday regimens, partly because other workers have used it in analogous tests, and not least because it is considered by many to be the most noxious of fats in relation to the disease process under investigation.

The feeding of butter fat as butter under South African conditions of temperature presents difficulties. The unit of intake ultimately decided upon was the $5^1/_2$ tin (can) of Nestlés Cream, containing 31 to 34 gm. ($1^1/_4$ ounces) butter fat. In the performance of the tests, series of subjects (Bantu and white) consumed either a meal of canned fruit and bread rolls (the fat-free meal), or a meal of canned fruit, rolls and the tin of cream, blood being sampled before (about noon) and three hours afterwards, and the clot lysis times determined.

Unfortunately, it was seldom possible to persuade the same group of subjects to participate in both fat-free and fatty meal tests.

RESPONSE TO EXERCISE

In seeking to determine the part played by exercise in its effect on fibrinolytic activity, again there are two alternative courses: (1) Exercise, if vigorous, will demonstrate its role in an exaggerated manner in influencing fibrinolytic activity;[26–28] it has seemed to me much more desirable to learn of the role of exercise under conditions relevant to everyday life. It was therefore decided firstly to carry out a comparative test on groups of subjects, working at, or relaxing from their usual tasks; and secondly, in the case of subjects studied at this Institute, to determine whether an inverse correlation obtains between clot-lysis time and degree of habitual physical exercise, both for individuals and for groups of persons. In the carrying out of the comparative test a series of male and female Bantu adults at Kanye were divided into two groups, each consuming the fat-free meal at noon. One group "loafed" for three hours; the other group pursued their usual tasks (washing, cleaning and gardening). Blood was sampled at the appropriate times, and the clot-lysis times determined.

TABLE I
Blood Fibrinolytic Activity in South African Bantu and White Males

Population Group	Age (yr.), Mean and Range	No. of Subjects	Lysis Time (hr.), Mean and Range
Combined rural Bantu group..	35 (19–75)	118	3.17 (1.5 –8.0)
Johannesburg Bantu.....	35 (20–62)	93	3.81 (1.75–9.5)
White subjects..	33 (15–66)	44	4.42 (2.0–11.5)

OTHER STUDIES

Various biochemical, haematologic and other tests were undertaken on whole blood (also plasma and serum) from subjects. Although data on serum protein fractionation and on thymol turbidity are mentioned later, they will be discussed in detail in subsequent publications. A point worth mentioning at this juncture concerns the commonness of abnormally high erythrocyte sedimentation rates in the Bantu subjects.[29]

TABLE II

Blood Fibrinolytic Activity in South African Bantu and White Females

Population Group	Age (yr.), Mean and Range	No. of Subjects	Lysis Time (hr.), Mean and Range
Combined rural Bantu group.	34 (18–68)	131	4.53 (1.75– 9.5)
Johannesburg Bantu.....	33 (17–59)	56	4.74 (2.0 –10.0)
White subjects..	29 (16–65)	55	4.00 (1.75– 8.5)

TABLE III

Effect of Fat-Free and of Fatty Meal (31–34 gm. Butter Fat) on Mean Changes in Blood Fibrinolytic Activity of Bantu and White Subjects

Type of Meal	Bantu		Whites	
	Males	Females	Males	Females
Fat-free meal....	3.75→3.75 (40)	4.3→4.2 (49)	4.2→4.4 (29)	3.8 →4.1 (26)
Fatty meal.	3.65→3.45 (55)	4.4→3.95 (54)	4.3→4.1 (31)	3.65→3.9 (32)

NOTE: Mean lysis time in hours, at about noon and three hours later. Figures in parentheses denote numbers of subjects. Mean ages of all groups were similar, ranging from thirty to thirty-three years.

TABLE IV

Effect of Exercise on Blood Fibrinolytic Activity in Bantu Subjects

Data	Rural Bantu Males		Rural Bantu Females	
	Active	Not Active	Active	Not Active
Number of subjects.....	19	21	20	18
Mean lysis time of blood sampled at noon.....	3.0	3.15	4.25	4.3
Mean lysis time of blood sampled at 3 P.M.....	3.1	3.15	4.15	4.4

NOTE: Mean lysis time in hours. Mean ages of groups were similar, ranging from thirty to forty years.

RESULTS

The results of these experiments are summarized in Tables I through IV.

Since the mean clot-lysis times for each sex in the three rural groups of Bantu studied (Shangaans, Pedi and Tswana) were found to be closely similar, results have been combined into a single group for each sex. *Table I:* Mean fibrinolytic activity of blood from rural Bantu males is significantly higher than that from urban Bantu males ($P<0.002$); mean activities for rural and urban Bantu male groups are significantly higher than for white males ($P<0.0005$ and $P<0.05$, respectively). *Table II:* Mean activities of blood from Bantu females, rural and urban, are significantly lower than that of white females ($P<0.05$ and $P<0.02$, respectively). In the Bantu, the sex bias of activity in favour of males prevails significantly in both rural and urban groups ($P<0.00001$ and $P<0.001$, respectively). There is no significant difference in mean activities between white males and females ($P<0.20$). *Table III:* Mean fibrinolytic activity determined in Bantu and white subjects (males and females) at noon, followed by a fat-free or fatty meal (31 to 34 gm. butter fat) reveals inconsistent and not significant changes; the majority of Bantu males and females tend to show acceleration of activity following the fatty meal; White females tend to show slight inhibition of activity after both fatty and nonfatty meals. (For the maximum change, i.e., increase in fibrinolytic activity in Bantu females on the fatty meal, $P = 0.20$.) *Table IV:* No significant difference in mean blood fibrinolytic activity is apparent between Bantu groups active and nonactive under the experimental conditions employed.

COMMENTS ON RESULTS

It is imperative to bear in mind the uncertainty over the relevance of fibrinolytic measurements carried out *in vitro* to conditions *in vivo*. It must be stressed that all subsequent comments and discussion refer exclusively to the results of the test tube studies.

Regarding repeatability of observations, findings in general are in agreement with those of Billimoria et al.[26] who found that activity

levels in blood do not usually vary grossly when sampled at the same time on different days. With fifteen of twenty subjects examined at the same time of day on six or more occasions, examples of sequences of lysis times (in hours) are as follows: (1) six and one half, seven and one quarter, seven and one quarter, six and three quarters, six and one half, seven; (2) three, two and three quarters, three and one half, three and one quarter, three, three and one quarter. on the other hand, in the other five subjects, examples of sequences of lysis times (in hours) are: (1) six, two and one half, five and one half, four and three quarters, six and one half, four; (2) three and three quarters, seven and one quarter, four, six and one half, five, six. Briefly then, it would seem that whereas fibrinolytic activity at noon is fairly constant with the majority of subjects, it is irregular with the minority. In the latter subjects, the etiological significance of these gross changes is doubtful in view of such fluctuations being found to be as common among Bantu as among white subjects. With plasma, in contrast to blood, Buckell and Elliott[25] have reported considerable irregularity in fibrinolytic activity in subjects examined on different occasions.

The superiority of fibrinolytic activity found in Bantu males compared with white males (Table I) was as expected, and confirms the conclusion reached locally, both for blood[16,20] and for plasma.[22,23] Our mean value for white males bled at noon, namely, 4.42 hours (Table I), may be compared with the mean for the ten men examined at 2 P.M. by Fearnley et al.,[30] namely, 4.6 hours. Our mean value for white males over thirty years of age, namely, 4.65 hours, is similar to that given by Nestel[31] for thirty elderly Australian white males, namely, 4.9 hours, although the time of collection of blood was not stated.

The superiority in mean blood fibrinolytic activity of white females over Bantu females was entirely unexpected. No explanation can be offered. Our mean value for young white females under thirty years of age bled at 3 P.M. and without respect to diet consumed (whether fat-free or fatty) was 3.85 hours. This figure may be compared with the value, namely, 3.7 hours, reported for the group of fifteen nurses (presumably in the younger age group) bled at 4 P.M. by Fearnley et al.[24]

Combining information given in Tables I and II, two comments may be made. (1) Merskey et al.,[20] in their comparison of Bantu and white males, have entertained the possibility that fear among the former regarding venipuncture, might augment their fibrinolytic activity.[32] That possibility was also considered at this centre, but was discarded for the following reasons. Firstly, of the Bantu (both rural and urban) who were bled on several occasions for lysis time determination, their becoming accustomed to the taking of blood samples had no obvious influence on the values obtained. Secondly, mean fibrinolytic activity of Bantu females is lower than that of white females (Table II.) (2) Gillman,[33] referring to the commonness of liver dysfunction and disease in the Bantu, considers that such conditions almost certainly have a bearing on the rarity of mortality from coronary heart disease among them. More apposite, Gillman et al.[33] have confirmed in Bantu the observation of Kwaan et al.[34] that higher fibrinolytic activity in plasma is usual in those who suffer from cirrhosis, compared with control subjects, an observation further confirmed in the present study using whole blood from both Shangaan and Pedi Bantu ambulant patients with cirrhosis (mean lysis time, 2.85 hours for ten male patients bled at noon). Moreover, Merskey et al[20] have noted liver dysfunction, as reflected by certain biochemical tests, to be more prevalent in Cape Town Bantu males compared with white males (as also is cirrhosis[35,36]), and have wondered whether the more rapid lysis times of Bantu may not be a manifestation of such dysfunction. At this centre, numerous investigations have testified to the commonness of liver dysfunction and disease among Johannesburg Bantu.[37–41] There is certainly no intention of excluding the possible role of such stigmata in influencing fibrinolytic activity: nevertheless, it may be pointed out that although certain of our Bantu groups (male and female) who

were investigated to shed light on this aspect, had higher mean serum gamma globulin and thymol turbidity levels and lower serum albumin levels compared with white subjects, no correlation was apparent in individuals between such data and fibrinolytic activity. In addition, although mean values among the Bantu for the serum components mentioned were similar for both sexes, there was a significant sex difference in mean fibrinolytic activity.

Among both races and sexes, and in every run of determinations, lysis times were accelerated in some subjects, whereas they became prolonged in others following the fatty meal. But broadly, Bantu males and females evince a tendency toward acceleration of fibrinolytic activity, acceleration occurring in roughly half of the subjects, little or no change in a quarter, the remainder showing inhibition. On the other hand, there is a tendency for slight inhibition of activity to occur with white females consuming either type of diet (fat-free or fatty), about half showing inhibition, a quarter little or no change, and a quarter showing acceleration. Mean changes, however, are not large (Table III) and it would seem prudent not to lay stress upon them, but simply to emphasize the inconsistency of the response. Nitzberg et al.[42] using the same method of determination, investigated the response to a meal containing 85 gm. animal fat by groups of normal, hyperlipemic, hypercholesterolaemic and coronary patients; after three hours a "definite postprandial shortening of fibrinolysis time" was observed in twenty-six of twenty-nine subjects. Whether such acceleration of activity was statistically significant or otherwise was not stated. Employing a modification of the Fearnley method on whole blood, Billimoria et al.[26] reported a general decrease in fibrinolytic activity following consumption of 42 gm. butter fat, this change being apparent in fourteen of twenty-one subjects after a two-hour interval (statistically significant,) and in ten of fifteen subjects after an interval of four hours (not statistically significant).

Turning now to plasma, using a modification of the clot lysis technic of Bidwell[43] as described by Biggs and MacFarlane,[44] Greig[28] found an inhibition of fibrinolytic activity in all subjects studied (twenty-six males and females) three hours after the consumption of a fatty meal made up of everyday foods, when judged over a twenty-four-hour incubation period. In addition, Greig and Runde[45] reported that inhibition was greater with butter fat and eggs, compared with the vegetable oils tested (corn, coconut, sunflower and arachis). Using these technics, Buckell and Elliott[46] studied the response (three hours later) to 50 gm. butter fat by twenty normal men; they found a decrease in activity only in eight subjects when determined after a six-hour incubation period, and in five of twenty when determined after a twenty-four-hour incubation period. Bradlow et al.[23] using the same method on plasma, studied a series of twenty-three Bantu and eighteen white subjects after an overnight fast, and four hours after ingestion of a fatty meal; no significant change in fibrinolytic activity was found. Hougie and Ayers,[47] working with their own modification of the method on plasma, were unable to confirm that lipaemia inhibits "fibrinolysis potentiality." Their research indicated that the ingestion of butter, cream and eggs had no significant effect on "fibrinolysis potentiality" determined between three and one half and four hours after the meal. The foregoing, and other studies that could be cited, thus reveal complete disagreement (both for whole blood and plasma) over the effect of a fatty meal on fibrinolytic activity, in which increase, little change and decrease in activity all have been reported. It would not be appropriate at this juncture to seek to elucidate the reasons for disagreement, but in judging the lack of concordance in observations, the following must be remembered: (1) the existence of the diurnal rhythm of fibrinolytic activity and the different times of collection of blood samples; and (2) the differences in methods of determination, and also the fact that some studies were undertaken on blood and others on plasma.

Before considering the effect of exercise on fibrinolytic activity, attention must be drawn to the inexplicably high level of motor fitness of the Bantu (children, young men and older men) as assessed by the Harvard step test,[48]

a superiority out of proportion to the admittedly higher level of habitual physical activity common to these people. Attention is also drawn to the fact that in rural areas, the harder physical work (housework, fetching water, "stamping" or grinding of maize and weeding) is normally carried out by the Bantu women. Yet, fibrinolytic activity in Bantu females has been found to be *lower* than that of Bantu males, and lower too than that of the white women studied (Tables I and II). Table IV reveals that the two rural Bantu groups investigated under different conditions of bodily activity showed no difference in mean fibrinolytic activity, i.e., it would seem that the relatively leisurely physical activity of the "active" Bantu studied was insufficient to accelerate their blood clot lysis times. Furthermore, examination of individual fibrinolysin values for Bantu and white subjects investigated at this Institute has shown no correlation with level of individual habitual physical activity.

There is no doubt, of course, that under conditions of physical activity divorced from that experienced habitually, there is enhancement of fibrinolytic activity. This has been demonstrated in plasma of subjects after running up and down stairs, although the level returned rapidly to normal soon after cessation of exercise.[27] Enhancement of activity in plasma was also noted by Greig,[28] and in whole blood by Billimoria et al.[26] following periods of brisk walking; the latter group also demonstrated the evanescent character of the stimulated fibrinolytic activity. The somewhat negative findings reported in this section do *not imply* that the higher motor fitness of the Bantu is of little relevance to their freedom from death from coronary heart disease. Observations merely indicate that within the limits of the sensitivity and specificity of the test employed, marked differences in fibrinolytic activity associated with differences in *habitual* exercise have not been demonstrable.

Not apparent from the tables are a number of interesting points. (1) Among both Bantu and white subjects, lysis times of as long as six to eight hours were encountered occasionally in young persons; on the other hand lysis times of two to two and one half hours were noted not infrequently among very elderly subjects. (2) As already state, most subjects have similar lysis times when examined on several occasions at the same time of day. Furthermore, activities measured in *groups* of subjects have shown higher activity early in the morning and a roughly steady state from noon until late afternoon. Nevertheless, both time of peak activity and amplitude of diurnal rhythm have been found to vary from subject to subject.

COMMENTS

All that I wish to conclude and discuss from the experimental observations reported is the superiority of fibrinolytic activity in Bantu men, the inferiority (or at least lack of superiority) in Bantu women, and the inconsistent response to a fatty meal and to exercise. In discussing the relevance of these findings to the wider subject of ischaemic heart disease, it is useful to consider the aspects of blood coagulation and fibrinolysis together.[49]

The conception of decreased coagulability and of increased fibrinolytic activity operating to obviate serious ischaemic episodes in a population such as the Bantu is plausible, and numerous schema have been advanced which imply the possibly important role of these two factors in the pathogenesis of arterial thrombosis.[14,21,50,51] However, the evidence bearing on the subject is far from conclusive. It should be noted that Poole[52] has emphasized that "It is still too often assumed that factors which influence clotting must necessarily influence thrombosis and conversely that factors which do not affect clotting cannot be concerned in thrombus formation. Such assumptions are not justified. Later work may well show that factors which are important in coagulation are unimportant in thrombosis and vice versa." Merskey[17] also has pointed out that "it is still unproved that there is *any* relationship between blood coagulation and coronary artery disease." Others[53] have averred that "There is no clear evidence that fibrinolysis plays any part in the normal working of the body."

The Bantu may be regarded as a critical population because they are characteristically free from mortality from coronary heart disease. What information then have they to contribute? In regard to blood coagulation, the studies of Merskey and co-workers[16–20] have failed to reveal that the Bantu are at an unequivocal advantage in comparison with the white population. The observations on fibrinolysis described in this paper certainly have confirmed that Bantu males have a superior capacity to lyse blood clots, but any intention of attaching etiologic significance to this observation is precluded by not finding similar superiority of activity in blood from Bantu females. The existence of this significant sex bias (not present in white subjects) is puzzling; in any case it is the reverse of what one would conjecture. There appears to be no sex bias of lesions in the aorta and coronary vessels of the Bantu,[7] nor apparently of deaths from coronary heart disease, few in number though they be.[8,54] If the foregoing be the confused position in regard to blood coagulation and fibrinolysis with a population not prone to die from coronary heart disease, is the corresponding picture at the other extreme, i.e., among those who have had a coronary episode, any more definite?

On the basis of their careful investigations on animals and studies on human autopsy patients, Thomas et al.[15] have postulated "that two factors are involved in arterial thrombosis: (1) a *local* factor (arteriosclerosis), and (2) a haematological factor (either antifibrinolytic or procoagulative or both)." Now some workers, for example, McDonald and Edgill,[55,53] have reported increased coagulability of blood in patients with ischaemic heart disease; but with other workers,[16,20,42,50] this phenomenon has not been apparent, or only to a limited extent. Although significantly decreased fibrinolytic activity of whole blood has been reported in elderly subjects with intermittent claudication,[31] a decrease in plasma fibrinolytic activity was noted in a series of patients with coronary disease *only* for a few days immediately after infarction.[57] Furthermore, Merskey et al.[16,20] have found no difference in mean blood fibrinolytic activity between normal white control subjects and patients who undoubtedly had ischaemic heart disease. In addition, no correlation was found between the level of fibrinolytic activity in patients with coronary disease and the clinical severity of infarction.[18] Briefly then, it will be apparent that in the sequence of Bantu, "normal" white subjects and white subjects with coronary disease, there is no nicely graded series with blood coagulation and with fibrinolytic activity, such as obtains with levels of biochemical components such as serum cholesterol.[38,58]

The information on the influence of a fatty meal on blood coagulation and fibrinolysis is discordant. Significant alterations in certain blood coagulation criteria evoked by the ingestion of a fatty meal have been reported by some workers,[59–64] but not by others,[20,42,65,69] except in the case of the "Stypven" time. As already referred to, an increase in fibrinolytic activity following consumption of a fatty meal has been reported by some workers, yet a measure of inhibition by others. In the assessment of such controversial evidence, different conclusions are being drawn. Thus some authorities, such as Jolliffe,[14] consider that "the increased blood coagulability and decreased fibrinolytic activity of certain fats seem established." Others,[42] however, seem driven to conclude that the effect of hyperlipaemia on coagulation and fibrinolysis may not be of major importance in the development of ischaemic heart disease.

The subject of physical exercise and blood coagulation seems to have been insufficiently studied. Billimoria et al.[26] found no change in "Stypven" times. There is no doubt, however, that vigorous exercise and brisk walking temporarily do accelerate fibrinolysis. Nevertheless, the observations reported herein indicate that differences in fibrinolytic activity, whether between groups or among individuals, do not appear to parallel differences in habitual physical activity.

The observations on the Bantu provided herein do little to clarify why these people do not die from coronary thrombosis; indeed, they bring to the fore a number of questions.

(1) A segment of the Bantu population have

relatively high serum cholesterol levels, long clot-lysis times, severe arterial lesions, also hypertension: why do not more of the elderly people in this particular group die from coronary thrombosis?

(2) Why do the antithrombogenic factors which presumably protect the Bantu from acute occlusive lesions in the coronary vessels fail to prevent them from serious disease or death from thromboembolic lesions occurring in other parts in their arterial system? Hartroft et al.[51] have discussed the hypothesis that thrombotic phenomena elsewhere in the body would be expected to be correspondingly low in populations in which coronary heart disease is rare. They adduce that among Uganda Africans the hypothesis is valid. The situation, however, with the South African Bantu would seem to be out of harmony, for cerebral vascular disease (including cerebral artery thrombosis) is an important cause of death.[2,9,10,70,71] Indeed, for the forty-five to sixty-four year old age group, mortality rate from this cause among Johannesburg Bantu appears to be one of the highest in the world.[2] It must be admitted, however, that the precise moiety of deaths due to cerebral thrombosis is not known; furthermore, as Brock and Gordon[72] have pointed out, there is no adequate data on these people to assess the incidence of cerebral accidents due to atherosclerosis *per se*.

(3) Directly linked with the foregoing is the question of the rationale of seeking, under certain circumstances, to increase fibrinolytic activity on a long-term basis. The need for means to accomplish this has been indicated by a number of authorities.[73,74] Yet the anomalies revealed by the data on the Bantu render the position confused. Thus, the mean fibrinolytic activity in elderly Bantu men is much the same as that for young white females, yet elderly Bantu men, while not dying from coronary thrombosis, die readily from cerebral vascular disease. Again, the mean activity of elderly Bantu women is lower than that of elderly white women, yet the former, while not dying from coronary thrombosis, die readily (more so than men) from cerebral vascular disease; elderly white women die readily from both causes. In seeking, therefore, to raise fibrinolytic activity, whether therapeutically[75,76] or by means of restriction of diet and increase in exercise as indicated by Elliott,[74] what order of clot-lysis times are to be aimed at? In trying to answer this question, on the one extreme, ignorance of fibrinolytic activity in subjects just prior to an acute thrombotic episode provides a handicap which will not be easy to surmount; on the other extreme, the data on the Bantu, at least in respect to females, afford no guidance.

(4) If atherosclerosis stems primarily from successive thrombotic episodes, as the hypothesis of Duguid[77,78] suggests, one wonders why the progression of events in the Bantu almost invariably stops short of coronary occlusion?

It must be made abundantly clear that all that has been adduced and discussed herein does *not* imply that the *in vivo* processes of blood coagulation and of fibrinolysis, or the metabolic ramifications of fatty meals or of habitual activity, are of limited importance in regulating the development of ischaemic heart disease. It is entirely possible, as numerous others have stated, that present experimental technics fail to bring out all the differences that prevail between populations prone and not prone to die from coronary heart disease. But on the other hand, again quoting Merskey,[17] the possibility cannot be ignored that in such contrasting populations we are looking for differences in certain specific criteria which in actuality do not exist, or exist only to a limited extent.

SUMMARY

Fibrinolytic activity in whole blood has been determined in groups of rural Bantu, urban Bantu and white subjects. It has been found that: (1) Mean activity in rural Bantu males is significantly greater than in urban Bantu males; both groups are significantly superior in activity to the white males studied. (2) Rural and urban Bantu females have significantly lower activities than the white females studied. (3) Fibrinolytic activity determined in groups of Bantu and white

subjects at noon, followed by a fat-free or a fatty meal (31 to 34 gm. butter fat), shows no significant change when determined three hours later; in Bantu males and females there is a tendency toward acceleration of activity following ingestion of the fatty meal, whereas white females tend to show a slight inhibition of activity following both fat-free and fatty meals. (4) A group of rural Bantu pursuing active occupations did not have greater mean fibrinolytic activity compared with another group doing no active work during the experimental period, nor was a correlation apparent in the numerous individual Bantu and white subjects investigated.

It is imperative to take account not only of the limitations of current methods of determining fibrinolysin activity, but also of our ignorance over the applicability of the results obtained to *in vivo* conditions. Nevertheless, the experimental *in vitro* observations reported herein, indicating superiority in fibrinolytic activity of Bantu males over white males, but not of Bantu females over white females, demonstrate that higher fibrinolytic activity is not characteristic of the Bantu population. Caution must therefore be exercised against assigning undue importance to fibrinolytic activity (as assessed by the test used) in retarding the occurrence of acute thrombotic episodes in the coronary vessels of these people.

ACKNOWLEDGMENT

While I prepared all clots and observed almost all lysis times, various laboratory assistants have participated in the study and acknowledgment is made to Misses K. L. Mortimer, A. E. Cousins, S. E. Bloem, E. E. Bülow, N. A. Gardiner, M. G. Smith, E. M. Coalter, and S. J. Reynolds.

Gratitude is expressed to Mr. W. Lutz, of the Department of Mathematics, Witwatersrand University, who was responsible for the statistical analysis, and to Dr. H. D. Barnes who collected all blood samples taken at this Institute. Many thanks for cooperation and help are due to Drs. W. L. M. Downing and A. H. Davies of the Jane Furse Memorial Hospital, Sekhukhuniland, and to Chief Sekwati of the Pedi Bantu tribe; to Dr. J. A. Hay of the Seventh Day Adventist Mission Hospital, Kanye, Bechuanaland, and to Chief Bathouen of the Bangwaketse Tswana tribe; and to Dr. A. Beuchat, of the Swiss Mission Hospital, Chichumbane, Mocambique.

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Folic Acid Metabolites in Whole Blood and Serum in Anemia of Pregnancy

G. Izak, M.D.,* M. Rachmilewitz, M.D.,† A. Sadovsky, M.D.,‡ B. Bercovici, M.D.,§
J. Aronovitch, M.Sc.,‖ and N. Grossowicz, Ph.D.¶

Anemia of pregnancy is prevalent in tropical[1,2] as well as nontropical countries.[3,4] The mechanism responsible for the development of this anemia is not clearly understood despite the numerous studies reported in recent years.[3–6] The classification of this anemia is usually based on morphologic criteria and the response to treatment. The development in recent years of microbiologic assays for the quantitative determination of substances necessary for normal hematopoiesis permits a new appraisal of these anemias. The microbiologic assays for vitamin B_{12} activity in body fluids have contributed to the understanding of the role of this vitamin in anemia of pregnancy. Few data, however, exist concerning the role of folic acid in anemia of pregnancy.

This report deals with folic and folinic acid determinations in whole blood and serum of anemic pregnant women, using three microbiologic assays simultaneously.

MATERIAL AND METHODS

Fifteen hundred women underwent hematologic examination at the time of delivery in the Obstetrics Department of the Hadassah University Hospital. They ranged in age from sixteen to forty-two years. Forty-three per cent were multipara women with six or more previous pregnancies, 24 per cent with two to four previous pregnancies, 26 per cent with one to two previous pregnancies and 7 per cent were primipara. The majority of these women (78 per cent) came to Israel from Middle Eastern§§ and Mediterranean†† countries, while 22 per cent originated from Central and Eastern Europe.

Among the 1,500 women examined, 420 (28 per cent) were found to be anemic at the time of delivery (i.e., hemoglobin levels below 10 gm. per 100 ml.). In sixty-four of these, detailed folic acid studies were also carried out, using three different assays for the determination of the various forms of the folic acid group in whole blood and serum. A group of forty-three healthy subjects served as controls.

In anemic women the serum concentration of vitamin B_{12} was determined as well as a complete blood count including hemoglobin (cyanmethemoglobin), red cell, reticulocyte, platelet, white cell counts and hematocrit, according to standard procedures. Serum iron and unsaturated iron binding capacity were determined according to the method of Davis et al.[7] Serum vitamin B_{12} values were determined microbiologically, using a mutant strain of Escherichia coli as the test organism.[8] Whole blood and serum folic and folinic acid (citrovorum factor) were determined microbiologically. Lactobacillus casei was used for the determination of conjugated and free folic and folinic acids (which will be referred

From the Departments of Medicine B, Obstetrics and Gynecology, Hadassah University Hospital, and the Department of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

* Lecturer in Medicine; † Professor of Medicine; ‡ Associate Professor of Obstetrics and Gynecology; § Instructor in Obstetrics and Gynecology; ‖ Research Assistant, Department of Bacteriology; ¶ Associate Professor of Bacteriology.

This work was supported by grants from the Ford Foundation and from the International Hematology Research Foundation, Chicago, Illinois.

§§ Syria, Iraq, Lebanon, Iran, Yemen.
†† Tunis, Algeria, Morocco.

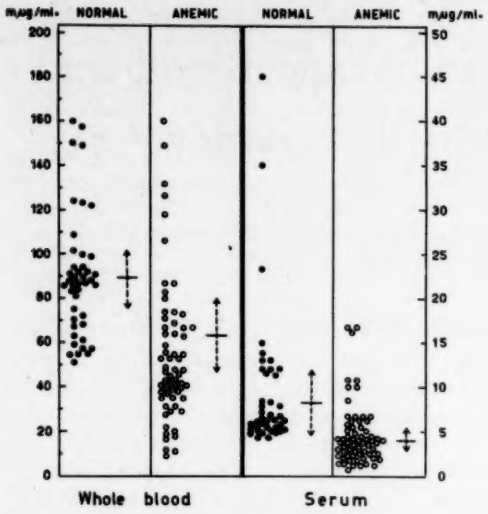

FIG. 1. Whole blood and serum total folic acid values in normal subjects and in anemic pregnant women.

to as "total folic acid"). Streptococcus fecalis was used for the determination of free folic and folinic acids and Pediococcus cerevisiae (Leuconostoc citrovorum) was employed to determine the free and conjugated folinic acid (citrovorum factor). The methods of the assay methods used are modifications of the procedures described by Toennies et al.[9] and will be published separately.[10]

RESULTS

The serum iron levels were found to be low, between 0 and 59 μg. per 100 ml., in fifty-two of the sixty-four patients (about 80 per cent) included in this study. The unsaturated iron binding capacity in these patients ranged from 290 to 476 μg. per 100 ml.

In thirty-six patients (about 50 per cent) the serum vitamin B_{12} level was found to be below 200 μμg. per ml. as compared to the normal average of 350 μμg. per ml. The serum vitamin B_{12} values ranged from 50 to 100 μμg. per ml. in fourteen of these patients, 100 to 150 μμg. per ml. in eleven and 150 to 200 μμg. per ml. in the remainder.

Folic Acid Studies

Normal Values. As seen from Table 1 the highest folic acid values were obtained in the whole blood of normal subjects when the microorganism used for the determination was L. casei (mean value 89 mμg. per ml.), as this organism utilizes in addition to pteroyl glutamic acid and folinic acid, di- and triglutamates of pteroyl glutamic acid (conjugated forms of folic acid). Much lower values (mean value 12 mμg. per ml.) were obtained with Strep. fecalis, as this organism responds to pteroylglutamic acid and folinic acids, whereas it cannot utilize pteroylglutamic acid conjugates. The values obtained with L. citrovorum, which measures only folinic acid and its conjugates, were about half of those found with Strep. fecalis (mean value 6.34 mμg. per ml.).

It can be seen that the bulk of folic acid is found in the erythrocyte. Employing L.

TABLE 1

Mean Normal Folic and Folinic Acid Values in Normal Subjects and in Women Found Anemic at Time of Delivery

Subjects	Total Folic Acid (L. casei) (mμg./ml.)				Pteroylglutamic Acid and Folinic Acid (Strep. fecalis) (mμg./ml.)				Folinic Acid (L. citrovorum) (mμg./ml.)			
	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum
	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±
43 normal subjects.	89.0	13.4	8.26	3.51	12.0	7.31	0.75	0.60	6.34	4.83	0.30	0.22
64 anemic women..	62.4	16.2	4.16	1.14	9.7	8.87	0.70	0.89	5.2	4.99	0.35	0.24

casei the whole blood contained 89 mμg. per ml. folic acid, while less than 10 per cent of this amount was found in the serum with the same microorganism. An even higher proportion of intraerythrocytic folic acid was found with the other two microorganisms, where the ratio of whole blood folic acid over the serum folic acid amounted to about 20.

Folic Acid Values in Anemic Pregnant Women. Total folic acid values in whole blood and serum are significantly lower in this group than those of the control subjects (mean value 62.4 mμg. per ml. for whole blood and 4.16 mμg. per ml. for serum) (Fig. 1). This difference was not apparent when the organisms used in the assay procedures were Strep. fecalis or L. citrovorum (Table I). The whole blood and serum total folic acid values varied widely in the group with anemia, hence the relatively high calculated mean value. In thirty-five of the sixty-four anemic women (about 50 per cent) whole blood and serum total folic acid values were very low and did not reach the lowest values obtained in any of the normal control subjects (i.e., 50 mμg. per ml.). Of these thirty-five women, seven had whole blood total folic acid values below 20 mμg. per ml., fifteen had values below 40 mμg. per ml. and thirteen below 50 mμg. per ml. Correspondingly low values were found in the serum of these women.

As can be seen from Table II, the simultaneous finding of low blood and serum total folic acid, low serum iron and low vitamin B_{12} was frequently encountered. In fourteen women these values were definitely below normal. In some patients only serum vitamin B_{12} and serum iron values were low, whereas folic acid levels were normal. The most frequent single deficiency was that of iron, encountered in thirteen women, whereas low values of folic acid only or vitamin B_{12} only were found in a few instances.

TABLE II

The Distribution of "Total Folic Acid," Serum Iron and Serum Vitamin B_{12} Levels in Sixty-Four Pregnant Anemic Women

Women (no.)	Total Folic Acid (mμg./ml.)	Serum Iron (μg./per cent)	Serum Vitamin B_{12} (μμg./ml.)
14	9.6– 48.0	13- 52	50–185
6	39.0– 49.0	66–109	50–200
13	12.0– 48.0	0- 49	248-450
12	74.0–132.0	10- 52	50–200
13	79.0–149.0	20- 47	200–421
4	81.0–106.0	70–109	50–145
2	11.0– 28.0	65–110	260–390

COMMENTS

The data presented here indicate that significantly low total folic acid values, as determined by L. casei, were found in whole blood as well as in serum in about 50 per cent of women found anemic at the time of delivery. These low folic acid values were frequently associated with low serum iron and/or serum vitamin B_{12} concentrations. No correlation was evident between the decrease in these hematinics in the blood and the type of anemia. A characteristic hyperchromic macrocytic anemia was found in only seven of these patients. In the majority (56 per cent), however, a dimorphic (macrocytic, hypochromic) type of anemia was seen. This lack of correlation is not surprising in view of the frequent association of several deficiencies, and corroborates our findings on the combined vitamin B_{12} and iron deficiencies in anemia of pregnancy, in which the presence of iron deficiency masks the morphologic characteristics of vitamin B_{12} deficiency.[11] The examination of the bone marrow also fails to supply definite criteria for the classification of these anemias. In patients with very low folic acid or vitamin B_{12} concentrations megaloblasts, when present, were found only in small numbers and at times were atypical (so-called transitional megaloblasts[12]), not justifying the widely used term megaloblastic anemia.

The frequent development of iron deficiency in pregnancy is a universal finding. The factors responsible for the depletion of vitamin B_{12} in pregnant women have been studied in recent years[11,13,14] and it has been established that various degrees of depletion are more frequent than was considered on the basis of morphologic criteria. The so-called megaloblastic anemia of pregnancy is known to re-

spond more readily to treatment with folic acid than to vitamin B_{12}.[15,16] The reason for this observation is not clear. Folic acid deficiency in anemia of pregnancy as manifested by low blood folic acid values on direct determinations has been reported in only a few instances.[17,18] This may be due to the fact that in these studies[17,18] only serum folic acid was determined. However, Toennies et al.[9] and our own findings showed that the bulk of folic acid is present in the red cells and only about 10 per cent of this vitamin is found in the serum. It is not surprising, therefore, that low folic acid values were not found to be associated with clinically suspected folic acid deficiency states, particularly with anemia of pregnancy. Indirect methods to detect folic acid deficiency were developed by Girdwood[19] and later by Chanarin et al.[20] These methods are based on diminished excretion of folic acid in the urine following an oral load and rapid clearance of the vitamin from the serum following its intravenous injection to folic acid-deficient patients as compared to normal control subjects. Using the latter procedure, Chanarin and associates[20] concluded that anemia of pregnancy is frequently associated with folic acid deficiency.

Baker,[21] Herbert and their associates[22] recently reported results obtained on folic acid determinations in the serum using L. casei. The normal range found by them was 7.5 to 23 mμg. per ml. This range is comparable to that obtained in our laboratory, which was 4.5 to 24 mμg. per ml. serum. The same authors found low serum values of both vitamin B_{12} and folic acid in two patients with megaloblastic anemia of pregnancy.[23]

As to the cause of folic acid deficiency, it is difficult to believe that only insufficient intake is responsible for the development of folic acid deficiency in anemic women, in view of the abundance of foodstuffs rich in folic acid (fresh vegetables and citrus fruit) or in factors activating folic acid in this country. In search of another or an additional cause, folic and folinic acid values were compared in paired fetal and maternal blood samples and it was found that fetal blood contains eight times more folinic acid (citrovorum factor) than its maternal counterpart.[24] It seems of interest that while the difference was found to be marked between maternal and fetal folinic acid values, the total folic acid in fetal blood was only about twice that of the maternal blood. This finding together with the decrease in whole blood total folic acid values in anemic pregnant women suggests that conjugated folic acid may be mobilized from maternal red cells finding its way through the placenta to the fetus where it is found mainly in its metabolically active forms.[24] It seems reasonable to conclude, therefore, that large fetal demand is mainly responsible for a relative folic acid deficiency in the mother, thus contributing to the development of anemia of pregnancy. Baker et al.[23] in their last report arrived at similar conclusions which are based on serum folic acid determinations of blood from a group of pregnant women and an unmatched group of cord blood samples.

SUMMARY

Folic acid determinations were carried out on whole blood and serum by means of three microbiologic assays. The total folic acid (conjugated and free pteroylglutamic acid and folinic acid) was determined by L. casei, free pteroylglutamic acid and folinic acid were determined by the use of Strep. fecalis, and P. cerevisiae was employed for the determination of folinic acid (citrovorum factor).

In sixty-four pregnant women with anemia, folic acid as well as serum iron and serum vitamin B_{12} levels were determined. In thirty-five of them, significantly low folic acid values were found in whole blood and serum when compared with values in normal subjects. Low folic acid values were frequently associated with low serum vitamin B_{12} and low serum iron concentration. The mechanism of the development of folic acid deficiency in pregnancy is discussed.

ACKNOWLEDGMENT

We are indebted to Miss R. Davidoff, M.SC., Miss F. Mandelbaum, M.SC., Mrs. M. Jablonska, Mrs. R. Pinstein and Mrs. B. Hutman for their very able assistance.

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A Nutrition Survey of the Armed Forces of Iran

JOHN H. BROWE, M.D., M.P.H., JOSEPH S. BUTTS, PH.D., JOHN B. YOUMANS, M.D., PHILIP L. WHITE, D.SC., C. FRANK CONSOLAZIO, DAVID B. HAND, PH.D., ARTHUR G. PETERSON, PH.D., COL. LAURENCE M. HURSH, M.D., BRIG. GEN. MORTEZA TADAYON, M.D., BRIG. GEN. SOLYMON DEYHIMI, M.D., D.V.M.,* COL. RAHMAPOLLAH DEYHIMI, D.V.M., MAJOR ALI KHAN AFRAKTEH, CYRUS E. FRENCH, PH.D.† AND ARNOLD E. SCHAEFER, PH.D.

THE food and nutrition situation in several countries in the Middle East underwent a preliminary survey in a two-week period in 1955.[1] Shortly thereafter invitations were received by the Interdepartmental Committee on Nutrition for National Defense (ICNND) for assistance in conducting complete nutrition surveys in Iran and Pakistan.[2] Field survey teams were recruited, all necessary supplies and equipment were assembled and the survey teams were dispatched on January 20, 1956. Dr. John B. Youmans, a consultant to the Committee, was Field Director of the surveys in both Iran and Pakistan. The late Dr. Cyrus E. French was Deputy Field Director for both countries and served as Survey Director in Pakistan.

The survey team included a nutritionist, two clinicians, a biochemist, a food technologist, an agricultural economist, a laboratory supervisor and two laboratory technicians. The Field Director of the surveys, Deputy Field Director, food technologist, agricultural economist, one clinician and the laboratory supervisor divided their time between Iran and Pakistan. In addition, seventeen Imperial Iranian Army personnel were assigned to work with the team and three chemists from the Food and Drug Laboratory, University of Tehran, worked in the laboratory (see Acknowledgments). The survey included clinical observations, biochemical analyses, collection of dietary information and food analyses, the assembling of information on agriculture and food production, and background information on health and nutritional status in general. This report describes the procedures used, the major findings and essential background information.

BACKGROUND

History and Government

Iran, the legendary cradle of the Aryan people, has been the crossroads between Oriental people and nations to the west for some 3,000 years. Civilizations of various invaders have left their imprints. The Iranian people have a keen appreciation of their cultural heritage of art and literature. Verses from Saadi and Hafiz, famous poets of the thirteenth and fourteenth centuries, as well as from Firdausi and Omar Khayyam of the eleventh century, are cherished by those

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* Gen. Deyhimi, who placed his laboratory and its facilities at the disposal of the survey team, died while this manuscript was in press. At the time of his death he was Director of the Animal Affairs Laboratory, Veterinary Corps, Imperial Iranian Army.

† Dr. Cyrus E. French, who was Deputy Field Director of the Iran survey, died January 3, 1960, while this manuscript was in preparation. Dr. French had served as Consultant to ICNND since 1955. At the time of his death he served as Chief, Expanded Nutrition, UNICEF, United Nations.

This study was prepared at the request of and in coordination with the Interdepartmental Committee on Nutrition for National Defense, Building 16A, National Institutes of Health, Bethesda 14, Maryland. Tables presented in this paper are abridgments or combinations of those given in the original report of this survey. For the complete presentation of tabular data compiled for this survey the reader is directed to the original report, available from the office of the ICNND.

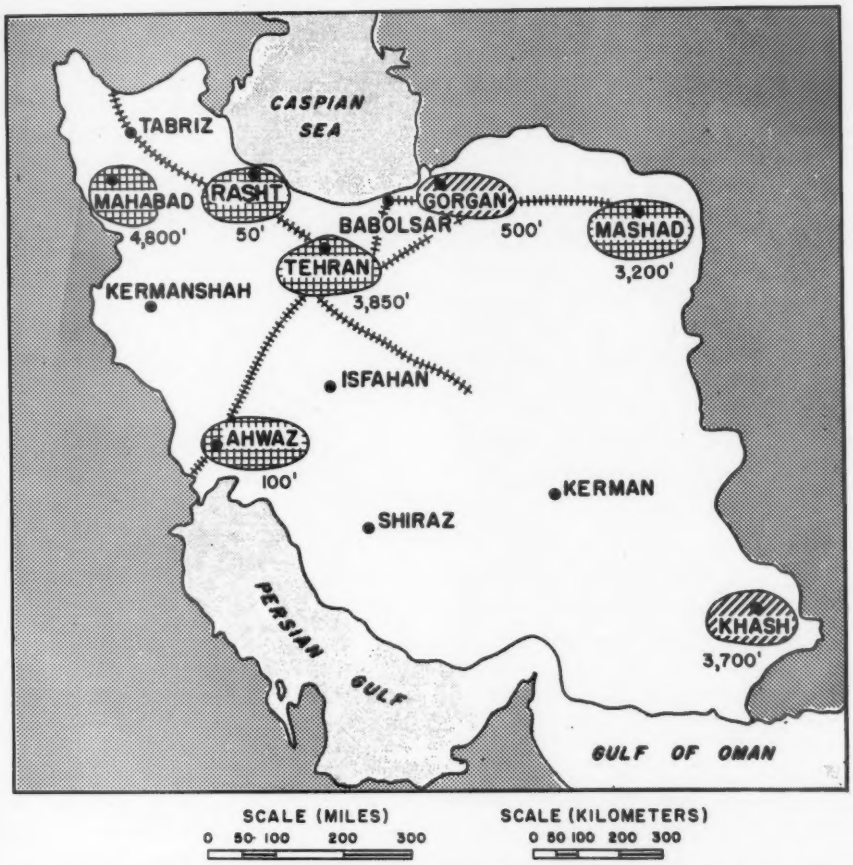

FIG. 1. Iran: nutrition survey areas. Original survey included five areas in crosshatch. Supplemental surveys by Iranian team were made in Khash and Gorgan. Figure by each shaded area indicates the altitude of the city.

in all walks of life, illiterate shepherds and peasants and the most learned alike.

Cyrus the Great unified the Medes and Persians about 550 B.C., laying the foundation for the first "world state" with his capital at Pasargadae. Darius the Great, chief architect of the Achaemenid empire stretching from Greece and Egypt to the Indus River, moved the capital south some fifty miles to Persepolis, near Shiraz, the first "world capital." The ruins of the great platform and buildings still stand. The spacious majestic monuments of Persepolis have been described as the greatest and most striking landmarks of Iran's brilliant past.[3]

After their conquest in the seventh century A.D. the Arabs transplanted the religion, culture and thought of Islam into the every day life of the Persians, where they have remained to a great extent to the present day. Seldom has a conquered nation undergone greater and more lasting changes and yet retained its separate entity.

In succeeding centuries Iran was invaded by waves of nomadic tribes from the east. Seljuk Turks, the Mongols of Genghis Khan, and Tamerlane all passed this way, and left their descendants among Iran's people. In the eighteenth century Quajar Turks became the ruling group, and remained in power until they were overthrown in 1925 by Reza Shah, father of the present Shah.

Iran became westernized during the time of the Quajars. The bazaar merchants, an influential group, demanded and received a constitution in August 1906. The next year Iran became a constitutional monarchy. Since then the National Assembly has taken over most of the authority and powers of government. The lower house, known as the Majlis, exercises considerably more power than the Senate, or upper house, which was not created until 1949. The Prime Minister is elected by the Majlis and appointed by the Shah. Some of the larger and well organized groups of tribes, such as the Kurds, Lurs, Bakhtiaris and Qashqais, exercise considerable self-government and have more national influence than their numbers alone would lead one to expect.

Geography

Iran is larger than Alaska and more than one-fifth the size of the rest of the United States. Lying south of the Caspian Sea and north of the Persian Gulf and Gulf of Oman, it is bordered by Iraq and Turkey on the west, Russia on the north and Afghanistan and West Pakistan on the east (Fig. 1).

About three fourths of the country is a great semiarid plateau from 2,000 to 8,000 feet in altitude, with many mountain ranges, narrow valleys, and two extensive deserts. Much of the desert area is too hot, dry and salty to sustain life. The plateau is bordered on the north by the Alburz Mountains which rise to nearly 19,000 feet on majestic Demavand. North of these mountains is the subtropical and fertile Caspian littoral and the Caspian Sea, now some 92 feet below sea level. On the west is the broad range of Zagros Mountains extending south and east. Beyond these mountains to the south is a narrow coastal plain bordering on the Persian Gulf and the Gulf of Oman. The country is divided into ten major political subdivisions known as Ostans.

The population of Iran is estimated at about 21 million and the annual rate of population growth at 1.5 per cent. This is a relatively sparse population and low rate of increase for Asia. Tehran, the capital, in north central Iran near the base of the Alburz Mountains at an elevation of about 4,000 feet, has a population of well over one million. Except for Ahwaz in the southwest, most of the major cities are located on the highland plateau. Aside from the larger cities, most of the people live in the north and northwest sections of the country. Nearly all of the rural population lives in some 7,000 villages.[4]

Agricultural Situation

Iran is largely an agricultural country. Eighty per cent of the people are engaged in crop and livestock production. Most foods are consumed by the families that produce them, the balance being sold or bartered in nearby areas, or sold in urban centers. The principal food product is wheat, although animal production is important. Little food is exported. The principal food exports are fruits (apricots, dates, raisins), rice, almonds and fish. Principal food imports are sugar and tea.

The agricultural potential of Iran is large if improved methods of irrigation, drainage, and water conservation, as well as crop and livestock practices could be applied. Mechanized farming is practiced in wheat and cotton growing on the Gorgan Plain and in some other areas. Cooperative machinery stations are increasing in importance. However, 90 per cent of the plowing in Iran today is done with a wooden plow.[5]

Livestock production is low, chiefly due to poor feeding, disease, and poor breeding. Many livestock have little but straw and weeds to eat for months at a time as a result of overgrazing.

Controlled grazing, seeding with high-yielding grasses, development of irrigated pastures, and production of hay and ensilage to supplement range grasses appear to be the more promising means to improve present conditions. One of the more significant recent developments in improvement of the feed situation is the salvage of some of the hundreds of thousands of tons of pulp (and some molasses) from sugar beet factories and the building of silos in the ground for storage. The installation of pulp-drying equipment at the sugar

mills—now being carried out on a small scale—will provide a much wider market for such feed, which cannot be transported economically in the wet form for more than a few miles.

Approximately 16 per cent of Iran's land is classed as agricultural, 10 per cent is arable, and 6 per cent is meadows and pastures. An additional 20 per cent, however, is potentially productive. As a rule, only one fourth of the arable land is in production in any one year, three fourths being left fallow to conserve moisture and build up soil fertility. One third to two fifths of the arable land is irrigated. Over two thirds of the land in cultivated crops is sown to cereals, chiefly wheat.

Most arable land is owned by absentee landlords. Oftentimes there are intermediaries who lease the land and sublease it to the tenants. As a rule farm produce is divided into five shares and distributed equally to those who furnish the land, water, seed, power, and labor. If the tenant furnishes only the power and labor, as is often the case, he receives two-fifths.

A program for the distribution of some of the Crown Lands, chiefly in the Gorgan Plain, has been in progress since 1953. Selected farmers are deeded from 5 to 20 acres of Crown Lands by the Shah.

Agricultural Programs

Various agricultural programs are being conducted jointly by the Government of Iran and the U. S. Operations Mission. Purebred cattle and sheep have been distributed through a dozen livestock stations and artificial insemination stations have been established. The introduction and crossing of Brown Swiss bulls with native cows has more than tripled milk production of the first generation crosses. Importation and crossing of Rambouillet rams with native sheep has markedly improved the quality of meat and the quantity and fineness of the wool.

A veterinary program of vaccination and parasite control is helping to reduce such common scourges as foot and mouth disease, anthrax and rinderpest. Slaughtering and meat handling practices are also being improved.

While New Hampshire Red chickens themselves cannot cope with the rigors of life in Iran, crossing them with the small native chickens has produced larger chickens and more eggs.

Improved seeds are being developed and disseminated.

Progress also is being made in the battle against locusts and some other crop and livestock pests.

The agricultural extension program initiated in 1953, public health programs, the Public Health Cooperative (described subsequently in the section on Health and Medicine) and the village aid program of the Near East Foundation are bringing practical education to the rural people.

The Near East Foundation is a private, nonprofit U. S. philanthropic organization which was established in 1930. Since 1941 it has been training Iranians to work in villages as teachers and agricultural extension and welfare workers. It gives technical assistance to improve agriculture and sanitation, and to encourage community development and adult literacy. In 1946 Iran invited the Near East Foundation to cooperate with various Iranian government ministries in a rural development program. Many of its programs are now financed by the Iranian government.

Systematic collection and dissemination of agricultural statistics began in 1955. Wholesale prices of major farm products in various cities of Iran are collected and published weekly. Retail prices and prices to producers are also collected. Crop reporting, market news services and market surveys are being developed. These programs are under the Department of Agricultural Economics in the Ministry of Agriculture.

Food Consumption and Production

The estimated per capita consumption of food for the entire population of Iran in 1954–1955 averaged about 1,967 calories per day, based on food balance data developed by the (U. S.) Foreign Agricultural Service. Sixty-nine per cent of the calories are obtained from cereals, largely wheat, along with some barley and rice. Animal products contribute 11.3 per cent, chiefly in the form of milk, mutton

TABLE I
Food Consumption in Iran, 1954–1955

Item	Consumption* (calories per person per day)	
	No.	Percentage of Total
Grains	1,358	69.0
Wheat	52.9	
Barley	9.1	
Rice (milled)	5.8	
Other	1.3	
Sugar	147	7.5
Fruits and nuts	106	5.4
Pulses	67	3.4
Oils and fats†	48	2.4
Vegetables	19	1.0
Meat	92	4.7
Mutton and goat meat	3.4	
Beef	0.8	
Poultry	0.5	
Milk	121‡	6.1
Eggs and fish	9	0.5
Total	1,967	100.0
Plant products	1,709	86.9
Animal products	258	13.1

NOTE: Source–Iran: Food Balance, 1954–1955, Foreign Agricultural Service.

* These data, "...refer to food consumed. They are not necessarily identical with amounts of food available for consumption because not all planted food crops are harvested, nor are all foods growing wild gathered."

† Three fourths of the oils and fats probably are of animal origin.

‡ Reduced from 155 to 121, June 1956, on the basis of revised estimates by the Foreign Agricultural Service.

and goat meat, with some calories coming from beef, poultry and ghee. Seven and a half per cent of the calories are from sugar, 5.4 per cent from fruits and nuts, 3.4 per cent from pulses, 2.4 per cent from fats and oils, and only 1 per cent from vegetables (Table 1).

Milk production in Iran is approximately one-half from cows, one-fourth from sheep, one-fifth from goats and one-eighteenth from buffalo.[6] Most of the milk is consumed in processed form and relatively little as fluid milk. "Mast" (a curdled milk resembling yoghurt) is commonly used. Considerable cheese of a soft, white type is made on farms by adding to milk an enzyme from sheep stomachs. Another milk product known as kashk consists of hard chunks of dried buttermilk which keep well and are readily reconstituted by soaking in water.

Ghee is normally a semisolid butter oil. In Iran fat from the tail of the fat-tail sheep is usually included. This tail, sometimes weighing 20 pounds or more, is practically all fat and serves as an energy reservoir for the animal. It is the chief source of fat for many Iranians. Iranians prefer animal ghee to vegetable ghee even at twice the price.

Citrus fruits are produced in considerable quantity in the Caspian region and in southern Iran. Oranges, lemons (sweet and sour), and tangerines are generally available in the cities and larger towns during the relatively short marketing season. In 1956 oranges averaged around 13 cents apiece in city retail markets. As they cost about one third of the daily wage of an unskilled laborer, they are too expensive for most of the population. Sun-dried limes keep well for two to three years. A considerable quantity of bottled lime juice, with fruit-pulp sediment, is produced and sold in the cities.

Transportation

More and better transportation is one of the greatest needs in Iran. The Trans-Iranian railroad runs from Khorramshahr, a port at the head of the Persian Gulf, through Ahwaz and Tehran to the Caspian Sea. The building of this railroad in the 1920's, principally by Swedish engineers, was a tremendous and difficult task. The railroad has 300 tunnels and rises to 8,000 feet above sea level. Tehran, Ahwaz and Mashad are the only major cities connected by railroad. Airplane service is available from Tehran to several cities, but inadequate runways, frequently unserviceable in stormy weather, make flying schedules uncertain. The only transportation for produce between some large communities is on the backs of men, donkeys and camels. Camel caravans are still an important means of transport in some areas.

Religion, Literacy and Language

Ninety-eight per cent of the Iranian people

are Moslems. The balance are Christians (chiefly Armenians and Assyrians), Jews, and Zoroastrians. Islam is the basis of morality and social order. In many villages the mullah, or Moslem priest, serves as the magistrate in settling disputes, and is an influential member of the community.

More than 80 per cent of the people are classed as illiterate, but considerable educational progress is indicated by the higher rate of literacy among children than among adults, particularly in urban areas. Only one fourth of the villages are reported to have schools. Six years of primary schooling (ages six to twelve) is legally compulsory, but oftentimes this cannot be realized.

Modern Persian is termed Farsi (from the Ostan of Fars, seat of the early Persian Empire). It has been written in Arabic script since the Arab conquest. Turkish is common in northwestern Iran and even among some tribes in the southwest. Kurdish is used rather extensively in the Zagros Mountain areas. There are also numerous other languages and dialects.

Health and Medicine

The most conspicuous progress in Iran in the last decade has been in the field of public health. Here, as in many other countries, relatively inexpensive newer methods of disease control have brought significant reductions in death rates. The Public Health Cooperative was established early in 1953 with substantial aid from the U. S. Operations Mission. Physicians and other specialists from the Ministry of Health work together with American counterparts in the Ostans and villages. This group has effected spectacular improvement in the control of malaria, which formerly caused many deaths. Outstanding work has also been carried out in recent years in health education, immunization and sanitation by this group and the Near East Foundation. There is a growing appreciation of the importance of a safe water supply to combat water-borne disease.

Despite progress in health and medicine, much remains to be done in health education, disease control, in particular of trachoma, and improvement in nutrition and sanitation.

Few studies have been made of the extent of dietary deficiency diseases, but physicians report deficiencies in proteins, vitamins, fats, minerals, and calories.[7]

A nutrition survey in two areas of Iran in 1951, during which 154 families reported what they ate for one week in each of four seasons and underwent physical examinations, reported that Iranian children were shorter and weighed less than those of the west, showed signs generally associated with malnutrition, and consumed insufficient animal protein, calcium and certain vitamins.[8]

The general impression gained from the many reports from Iranian doctors and nurses who have been interviewed by the authors of a survey conducted in late spring 1952, as well as from opinions of visiting medical observers, is that malnutrition is a serious health problem in most areas of the country, and also that there are definite seasonal variations in the degree of malnutrition.[9]

This study by Thomson and Mashayekhi[9] uncovered many gross signs and abnormalities which are generally associated with malnutrition states. Great differences were shown in the prevalence of these findings among different areas. The authors concluded that protein aud some of the vitamins, particularly vitamins A and C, and some members of the vitamin B-complex, were most likely to be deficient. In addition, it was reported in 1951 that not enough food was produced to meet the requirements of all the people, even if they could afford to purchase it.[10]

A rural health survey in 1954 of 1,965 households in fifty villages south of Shiraz indicated 4.4 persons per household, with an average of 1.3 rooms per family, or 3.4 persons per room. For dishwashing material, 90 per cent used soil and 72 per cent ash. Ninety-four per cent disposed of garbage in their yard. Forty-four per cent had no private or communal privy.[4]

Accurate mortality statistics are not available for Iran, but efforts are being made to improve this situation.

Certain demographic and health studies were made in 1948 and 1949.[11] These in-

cluded the questioning of some 1,600 women in rural areas who had had one or more pregnancies during the previous ten-year period. The following rates were calculated from these data:

Birth rate.....51 per 1,000 population
Stillbirth rate.....20 per 1,000 live births
Neonatal mortality rate.....
.....86 per 1,000 live births
Infant mortality rate.....
.....216 per 1,000 live births

"Quick" surveys were made in other areas between 1952 and 1955, and in 1955 and 1956 surveys were conducted in the northwest and in the Tehran area.[8,12]

The government medical school of the University in Tehran, which trains the great majority of doctors for the country, has a student enrollment of approximately 1,200. There also are medical colleges in Shiraz, Tabriz, and Isfahan. Several of the larger cities have one or more hospitals, public or private. There are two schools of nursing in Tehran.

ORGANIZATION

The American nutrition survey team arrived in Tehran on January 23, 1956, and remained in the country for about eighty-two days.

The nutrition laboratory was established in the Animal Affairs Department of the Imperial Iranian Army in Tehran, and served as headquarters for the American team. The Animal Affairs Department also supplied two laboratory helpers and three dishwashers.

Since one of the objectives of the survey was to train Iranian counterparts in the procedures employed, eight Iranian Army physicians, five officers from the Animal Affairs Department (Veterinary Corps), one officer from the Quartermaster Corps, and two members of the Food Service Branch of the Imperial Iranian Army participated intimately in every phase of the survey. In addition, and at the suggestion of officials at the University of Tehran, three chemists from its Food and Drug Laboratory worked alongside other Iranians and Americans in performing the routine analyses in the Laboratory in Tehran.

The nutritional assessment phase of the survey consisted of three parts: (1) a dietary (food intake) study, (2) physical examinations, and (3) laboratory studies. To assist in future planning of the ration, the food production potentialities and food technological practices were observed.

Selection of Sample

The Imperial Iranian Army is organized to consist of training regiments plus regiments which have received specialized training, i.e., combat or service regiments. Thus an installation is normally made up of a training regiment, a combat regiment, and service troops and technicians. All the men in the training regiment are taken in at one time and trained for six months, at which time they become combat troops. Enlisted men serve two years. Commissioned officers, and noncommissioned officers to a large extent, are career men.

The company was the basic sample unit. Companies with different activities were examined. The sample included medical detachments, infantry, artillery, cavalry, engineers, and military police.

Troops often serve in areas other than those from which they come. However, area of origin has not been considered in the statistical analysis.

In selecting the units to be examined, a careful survey of all available units was made, and all companies which were considered representative were given an equal opportunity to be chosen. The final selection was made by lot. The actual number of troops surveyed varied from place to place. The sample size was adequate to permit nutritional appraisal of the Army.

Six areas representative of the deployment of the Iranian Army and taking into account the effect of local conditions on food supply and the major geographic sections of the country were chosen as sites for the survey. The locations were as follows: Tehran in the north central area, Ahwaz in the southwest, Mashad in the northeast, Mahabad in the northwest,

Rasht in the Caspian area, and Khash in the southeast. Inclement weather prevented the U.S. members from surveying the troops in the Khash area. The Iranian members of the team carried out a survey of Khash after the departure of the U. S. team. Their observations are included in the results reported in this paper.

PHYSICAL EXAMINATIONS

Procedures

A general physical examination was made on a total of 2,027 men: 506 at Tehran, 308 at Ahwaz, 317 at Mashad, 307 at Mahabad, 292 at Rasht, and 297 at Khash.

Classification of general appearance was based on the subjective impression of the examiner. An examination of the head and neck was made with special attention given to the eyes, lips, tongue, and gums. Auscultation of the chest, palpation of the abdomen, inspection for edema, and elicitation of ankle reflexes were also carried out. Although a general examination was made in an attempt to appraise the general health of the population being studied, special attention was given throughout the examination to the skin, mucous membranes, and other areas in which nutritional deficiency signs frequently occur.

The Iranian Army physicians assisted in performing the physical examinations. These physicians first observed and later carried out the procedures, subject to review by the U. S. team physician. Each physician was assigned a particular body area for examination, and the duties were rotated so that each member gained the experience of conducting an entire physical examination. These same Iranian physicians performed the physical examinations in Khash after the departure from Iran of the U. S. survey team.

Identification, pertinent information and physical findings were entered upon the specially prepared McBee Cards at five examining stations in the manner generally prescribed in the 1957 edition of the ICNND "Manual for Nutrition Surveys."[13]

Such information as name, age to nearest year, time in service, unit and type of activity, area of origin by province and city and whether "rural" or "urban," suspected diseases (e.g., tuberculosis, trachoma, and malaria), and current diseases such as diarrhea was obtained by the Iranian physicians. Each man was given a number consecutively as he appeared for examination.

Height was obtained without shoes, with the subject standing erect against a wall. It was read to the next lowest quarter inch, from a chart marked in quarter inches. Weight was obtained in pounds with subjects wearing only undershorts. The appropriate "standard weight" for height was later entered from the Davenport tables,[14] from which weight groups by "per cent of standard weight" were set up in intervals of 10 per cent.*

The first clinical examination consisted of examination of the chest, abdomen, and lower extremities. Procedures conformed to those described in the 1957 edition of the "Manual for Nutrition Surveys"[13] with the following exceptions: Auscultation of the chest was carried out with special reference to cardiac murmurs, but pulse and blood pressure were not taken unless cardiovascular disease was suspected. The lower extremities were palpated for edema and calf tenderness. Loss of vibratory sensation was tested for at malleoli and mid-tibia of both legs. Loss of ankle and knee jerks were recorded only when bilateral and reinforced. Position sense of toes was ascertained only if knee jerks and vibratory sense were absent. Plantar dysesthesia was sought by drawing the handle of the percussion hammer lengthwise along the soles of both feet.

The next station was ordinarily manned by two pairs of Iranian physicians, one of each pair examining and the other recording, and alternating to share the procedures equally. Examiners here were concerned with general appearance of the subject, which was based on the subjective impression of the examiner of a healthy male, and on those anatomic areas in which physical signs usually associated with

* These tables present graded average weight in pounds of men of different statures at various ages. Calculations are adjusted for shoes and clothing.

nutritional deficiencies occur. Alopecia was noted only when more than half of the head was bald, presumably due to favus.

The earliest sign of nasolabial seborrhea was considered to be filiform excrescences. These had to be plainly evident on inspection, and more evident after rubbing. When greasiness and redness were present alone in the nasolabial folds, the presence of seborrhea was not indicated. Malar erythema and infraorbital pigmentation were not recorded.

In order for a finding of thickened conjunctivas to be recorded the following criteria had to be fulfilled: the bulbar conjunctival thickening had to be generalized in the nasal and temporal zones of both eyes, vascularity had to be generally increased, lack-luster present and scleral blueness completely absent. Pingueculae were not recorded. Redness of lids was a prerequisite for the recording of blepharitis. Softening of the cornea was looked for by observing the reflections of the head mirror on the subject's cornea. If the reflection was not distorted, softening was considered to be absent.

Geographic tongue was defined as a multiplicity of creases which gave the tongue a relief-map appearance. For this sign to be recorded walls and bottoms of the creases or furrows had to have papillae on them, and be generally apparent over the entire dorsum. Serrations were described as knife-like slashes on margins and sides of tongue; they were large, deep fissures.

Dental caries were recorded as slight when one or two decayed or filled teeth were noted; as marked, when almost all, or all except one or two, were decayed or filled; as moderate, when between slight and marked. Malposition was recorded when more than one or two teeth were out of regular alignment.

Symmetrical, thickened pigmented pressure points were not recorded. Bluish red, cold extremities were recorded when hands presented this appearance and felt cold, regardless of appearance or temperature of feet. Skin color blanched when pressure was applied.

Skinfold measurements were taken only at the right posterior surface of the arm and over the angle of the right scapula.

TABLE II

Per Cent "Standard Weight,"* Per Cent of Troops by Location

Location	No. Examined	<90%	90–109%	>109%
Tehran.....	506	7	79	14
Ahwaz.....	308	16	78	6
Mashad.....	317	18	75	7
Mahabad...	307	17	79	4
Rasht.....	292	10	75	14
Khash.....	297	45	54	1
		(18)†	(74)	(8)

* Based on U. S. age-height-weight averages. See reference 14, and comments.

† Figures in parentheses in this and subsequent tables represent averages.

TABLE III

Skinfold Thickness of Iranian Troops by Location

Location	No. Examined	Arm (mm.)	Scapula (mm.)
Tehran.....	506	7.57	6.13
Ahwaz.....	308	6.45	4.98
Mashad.....	317	7.44	5.96
Mahabad.....	307	6.82	5.51
Rasht.....	292	8.21	7.07
Khash.....	297	6.37	5.19
		(7.18)	(5.83)

A sixth step was incorporated into the procedure after the first hundred soldiers were examined in Tehran, in order to check examinations more efficiently. The U. S. team physician examined the card for completeness, obvious errors, and re-evaluated the subjects for evidences of lesions associated with nutritional deficiency to check these findings. Tendencies to overcall or undercall were thus picked up, and consultation with the Iranian physicians resulted in a decrease in variability in calling and grading lesions to at least the range of variation of one U. S. team physician.

Results

No subject was found to be suffering from far-advanced nutritional deficiency disease. Practically no evidence of gross caloric or protein deficiencies was found.

Seventy-four per cent of all troops were within the 90 to 109 per cent range of "standard

TABLE IV
Per Cent Prevalence of Selected Clinical Signs by Location

Clinical Signs	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Average
Eyes							
Thickened conjunctivas	7.1	7.1	15.5	7.2	8.6	24.3	11.3
Skin							
Follicular hyperkeratosis	21.5	21.4	30.3	49.5	43.1	46.1	32.2
Nasolabial seborrhea	4.5	3.6	1.6	1.0	0.3	9.8	3.6
Lips							
Angular lesions	19.0	7.5	13.9	18.9	10.6	11.1	14.1
Angular scars	20.0	18.2	17.0	19.9	29.8	29.3	22.0
Angular scars and lesions	16.0	1.6	2.5	4.2	5.1	0.3	6.1
Angular lesions + angular scars and lesions	35.0	9.1	16.4	23.1	15.1	11.4	20.2
Angular scars + angular scars and lesions	36.0	19.8	19.5	24.1	34.9	29.6	28.1
Tongue							
Fungiform atrophy	18.8	7.1	17.4	23.1	22.3	17.8	17.8
Papillary hypertrophy	10.1	5.2	9.5	15.0	4.1	8.4	8.9
Filiform atrophy*	9.5	2.3	5.4	6.5	8.2	5.0	6.6
Gums							
Marginal redness	24.7	12.3	9.8	7.8	6.5	21.2	14.8
Marginal swelling	38.5	18.5	22.1	13.7	5.1	23.6	22.2
Bleeding, "scorbutic-type"	20.6	12.7	13.2	7.5	5.5	16.8	13.5
Recession	72.7	51.9	50.5	39.1	46.2	41.1	52.5
Extremities							
Loss of ankle jerk (bilateral)	1.5	3.5	0.6	0.0	0.6	1.0	1.3
Edema of legs	1.4	0.6	0.0	0.0	0.3	0.3	0.5
Bluish cold extremities	90.0	26.6	71.6	88.6	84.6	8.8	64.8

* Findings of slight filiform atrophy not included.

weight," while 8 per cent were above and 18 per cent were below this range (Table II).

Skinfold thickness at arms and scapulas correlated with per cent "standard weight," r 0.39 and 0.24, respectively ($P < 0.01$), as did general appearance with per cent "standard weight" at the same level of significance (Table III). It is noteworthy that all skinfold thicknesses measured at the scapula are less than the minimum ICNND standard of 8 mm.

Only 0.5 per cent of those examined showed edema of the lower extremities, which is not a large enough proportion to suggest a general protein insufficiency (Table IV). Loss of ankle reflexes occurred in 1.3 per cent of the troops. However, an appreciable number of men examined showed physical signs which have been associated with less than optimal intakes of certain vitamins. This was true for vitamin A, with 32 per cent showing follicular hyperkeratosis, and riboflavin, with 14 per cent exhibiting lesions at the angles of the mouth and 4 per cent having nasolabial seborrhea. Vitamin C insufficiency was also suspected, since 14 to 22 per cent showed bleeding gums or marginal redness or swelling, although only 2 per cent of these were suggestive of "scorbutic-type" gums.

These generalizations apply to the total body of troops examined. However, there was considerable variation in the prevalence of physical signs in different geographic locations and some variation with length of service. There was also some variation between units of troops.

The prevalence of angular lesions was greatest in Tehran (35 per cent) and Mahabad (23 per cent, Table IV), and least in Ahwaz (9 per cent) and Khash (11 per cent). Men in service for less than two years appeared to have more acute lesions than those with more than two years service (Table V). Engineers (26 per cent) had the highest prevalence and artillerymen (12 per cent) the least (Table VI). Angular scars, presumably indicative of previous angular lesions associated with insuffi-

TABLE V
Per Cent Prevalence of Clinical Signs by Time in Service

Clinical Signs	0–6 Mo.	7–12 Mo.	13–24 Mo.	3–7 Yr.	>7 Yr.
Skin					
Follicular hyperkeratosis	27.8	41.7	39.4	8.0	5.5
Nasolabial seborrhea	4.8	2.0	4.0	1.6	1.6
Lips					
Angular lesions	13.7	14.1	17.8	3.2	4.7
Angular scars	24.3	21.5	22.3	15.0	17.3
Gums					
Marginal redness	19.2	10.9	15.5	7.2	8.7
Marginal swelling	26.1	18.0	24.0	10.4	16.5
Bleeding, "scorbutic-type"	16.9	10.1	15.4	4.8	5.5
Extremities					
Bluish cold extremities	66.9	68.1	68.2	58.4	30.7

TABLE VI
Per Cent Prevalence of Clinical Signs by Service Activity (Unit)

Clinical Signs	Infantry	Infantry (Training)	Artillery	Military Police	Engineers	Cavalry	Medical
Eyes							
Thickened conjunctivas	13.0	10.2	10.8	6.7	8.9	5.4	17.6
Skin							
Follicular hyperkeratosis	39.4	25.8	38.4	15.1	48.2	8.1	27.8
Nasolabial seborrhea	4.7	4.6	0.5	0.8	1.8	0.9	0.0
Lips							
Angular lesions	16.5	15.4	9.4	9.2	18.8	4.5	12.0
Angular scars	20.5	13.9	21.3	25.2	26.8	58.6	26.9
Angular scars and lesions	5.7	6.1	2.4	15.1	7.1	9.9	3.7
Angular lesions + angular scars and lesions	22.2	21.5	11.8	24.3	25.9	14.4	15.7
Angular scars + angular scars and lesions	26.2	20.0	23.7	40.3	33.9	68.5	30.6
Tongue							
Fungiform atrophy	19.4	12.4	21.0	18.5	24.1	8.1	23.1
Papillary hypertrophy	8.0	6.7	12.6	9.2	13.4	10.8	8.3
Filiform atrophy*	7.6	4.6	6.6	7.6	5.4	9.0	5.6
Gums							
Marginal redness	11.9	16.7	8.4	21.8	11.6	36.9	18.5
Marginal swelling	21.9	26.5	10.5	34.5	18.8	33.3	14.9
Bleeding, "scorbutic-type"	13.7	19.5	7.7	10.9	10.7	9.0	12.0
Recession	51.8	55.1	39.5	64.7	41.1	83.8	48.1
Extremities							
Loss of ankle jerk (bilateral)	1.2	2.8	0.0	0.0	0.0	1.8	0.9
Edema of legs	0.5	0.4	0.3	2.5	0.0	0.9	0.0
Bluish cold extremities	57.0	53.4	73.1	99.2	87.5	95.5	59.3

*Findings of slight filiform atrophy not included.

ciency of riboflavin, were most prevalent in Tehran (36 per cent) and Rasht (35 per cent). High prevalence rates were also found at Khash (30 per cent) and Mahabad (24 per cent). Angular scars were also most prevalent in the cavalry (69 per cent) and military police (40 per cent). Nasolabial seborrhea was most commonly found in Khash (10 per cent), the prevalence being more than twice that in Tehran (5 per cent).

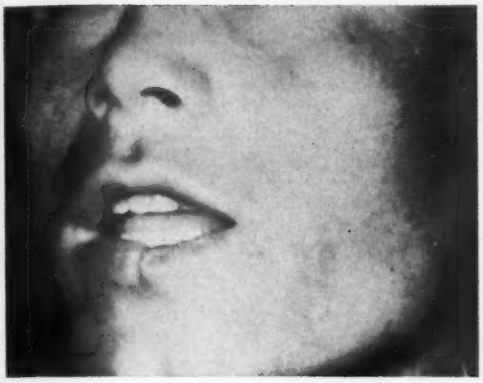

FIG. 2. Fissures at center of lower lip. These were seen in Tehran and said to be common. Similar to angular lesions in appearance.

A few soldiers seen in Tehran had a fissure at the center of the lower lip, similar in every respect to the angular lesions except for location (Fig. 2). The Iranian physicians stated that this was not uncommon, and occurs primarily in persons who live or spend much time in the open. Because of the marked similarity of these signs to the angular lesions, one soldier was given 10 mg. of riboflavin for ten days, and it was reported that the lesion had entirely cleared at the end of that time, even though the soldier had had the lesion for some months. On this basis, a suggestion was made to the Iranians that a controlled study be made with several persons presenting this lesion to determine whether or not the lesion could be definitively alleviated by administration of riboflavin.

Atrophy of the fungiform and filiform papillae of the tongue and papillary hypertrophy are signs suggestive of niacin deficiency. The greatest prevalence of these signs was found in Mahabad. Rasht and Tehran also were areas with high prevalence rates. The prevalence of these signs was least in Ahwaz.

Marginal redness, swelling, and bleeding of the gums were most prevalent in Tehran and Khash. The fewest number of persons showing these signs was found in Rasht. "Scorbutic-type" gums with bleeding were most commonly seen in infantry troops in training in Tehran. They were least common in persons with more than two years of service and among artillerymen.

Follicular hyperkeratosis at all body sites, which includes arms, back, thighs, chest, and buttocks, was most prevalent in Mahabad (50 per cent) and among engineers (48 per cent). It was much less common in persons with more than two years of service. It was least common in Ahwaz and Tehran and in the cavalry. Thickened conjunctivas, however, were noted most commonly in Khash and Mashad, and about equally (7 to 8 per cent), in the other four areas. This latter lesion was most common in medical troops, and least so among cavalry, military police, and engineers.

Although prevalence rates for loss of ankle jerk and edema of legs were not high, the former was most commonly encountered in Ahwaz, and the latter in Tehran, but edema of the legs was also noted in infantry training regiments and military police.

An interesting incidental finding was that of cold, bluish extremities, which occurred in 65 per cent of the men examined. While only 9 per cent of the troops in Khash and 27 per cent of the troops in Ahwaz showed this condition, it was noted in 91 per cent of the troops in Tehran and 89 per cent in Mahabad. Soldiers who had been in service more than six years showed this finding only 30 per cent of the time, as compared with 67 per cent of those with less than two years service. It was seen more frequently among cavalry and military police than among infantry and medical troops. The nutritional significance of this condition is unknown.

The distribution of soldiers whose body weights were within 90 to 109 per cent of "standard weight" ranged from 75 to 79 per cent in five of the areas, whereas in Khash it was only 54 per cent. The percentage falling into the less than 90 per cent "standard weight" category ranged from 7 to 18 per cent for five areas, with 45 per cent for troops in Khash. The number of persons over 109 per cent of "standard weight" ranged from four to fourteen of the sample, with less than 1 per cent of this group being from Khash (Table II).

Does this suggest that the standards of the Davenport tables, used for comparison in

TABLE VII
Per Cent "Standard Weight," Per Cent of Troops by Time in Service

Time in Service	No. Examined	Per Cent Distribution		
		<90%	90–109%	>109%
0–6 months....	629	15.5	76.0	8.6
7–12 months...	405	14.5	76.5	8.9
13–24 months..	741	12.7	77.7	9.5
3–7 years.....	125	38.4	57.6	4.0
>7 years.....	127	48.1	46.4	5.5
		(17.6)	(73.9)	(8.4)

TABLE VIII
Per Cent "Standard Weight," Per Cent of Troops by Service Activity

Service Activity	No. Examined	Per Cent Distribution		
		<90%	90–109%	>109%
Infantry.....	830	18.8	73.5	7.6
Infantry (training)....	461	19.1	72.3	8.7
Artillery.....	286	18.4	74.5	7.0
Military Police.....	119	5.0	77.3	17.6
Engineers.....	112	18.8	78.6	2.7
Cavalry.....	111	9.0	80.0	10.8
Medical.....	108	23.1	64.8	12.0
		(17.6)	(73.9)	(8.4)

this study, are too high for these people, or that a caloric problem may exist to some extent in all areas, but especially among the Khash troops? Study of these data arranged by time in service (Table VII) suggests that there is a tendency toward an increase in weight for height with time in service up to two years, which may be the result of an increase in calories consumed in the Army ration over that presumably consumed prior to conscription.

Data from those troops with more than two years' service suggest longer service tends to decrease weight. However, the trend to lower weight with time in service prompts the speculation that as men get older and assume family responsibilities, the economic burden may show itself as a weight loss, despite the possibility of obtaining more food outside of the ration. It also prompts the thought that part of the soldier's ration may be used in feeding the family, which could be done easily enough by bringing home a substantial part of the daily bread issue. On the other hand, the slight increase in number of those over 109 per cent of "standard weight" who are in service longer than seven years suggests these may include officers who may be better off financially, and hence better fed, or that noncommissioned officers with longer service have learned how to adapt better to the exigencies of military life than those with fewer years of service.

Table VIII presents the distribution of per cent "standard weight" by service activity.

Classification of general appearance of subjects into "good," "fair," "poor," or "cachexic," when compared with group per cent "standard weight," was significantly different from independent ($P < 0.01$), that is, appearance correlated very well with body weight, and hence with caloric status. Hence it can be reasonably stated that the subjects' caloric nutritional status played a major role in determining examiners' subjective impressions of their appearance. This is clearly evident in comparing the observations in Khash of distribution of per cent "standard weight" (Table II) and general appearance categories (Table IX).

Grouping general appearance by individual categories according to time in service, the distribution under "good" increases during the first year, and decreases somewhat in the second year of service. It again improves in the three-to-seven years service group, before declining again in the over seven years group. When "good" and "fair" are combined there is a progressive increase with time in service of per cent falling in this category until the over seven years group is reached, where percentage returns almost to that of the zero to six months group (Table X).

In general, the highest number of troops classified as poor or cachexic was found in the infantry training group (10.6 per cent), and the lowest was the artillery (3.8 per cent).

It is possible that other elements than posi-

TABLE IX
Per Cent Distribution of General Appearance by Location

Location	No. Examined	Good and Fair	Poor and Cachexic	Good	Fair	Poor	Cachexic
Tehran	506	95.1	4.9	40.9	54.2	4.9	0
Ahwaz	308	97.7	2.3	50.0	47.7	2.3	0
Mashad	317	94.6	5.4	47.0	47.6	5.4	0
Mahabad	307	93.2	6.8	47.9	45.3	5.8	1.0
Rasht	292	98.6	1.4	64.4	34.2	1.4	0
Khash	297	72.8	27.3	22.6	50.2	27.3	0
		(92.4)	(7.6)	(45.0)	(47.4)	(7.5)	(0.1)

TABLE X
Per Cent Distribution of General Appearance by Time in Service

Time in Service	Good and Fair	Poor and Cachexic	Good	Fair	Poor	Cachexic	Total
0–6 months	90.0	10.0	35.9	54.1	10.0	0	629
7–12 months	92.6	7.4	53.1	39.5	7.2	0.2	405
13–24 months	94.0	6.1	46.6	47.4	5.8	0.3	741
3–7 years	95.2	4.8	53.6	41.6	4.8	0	125
>7 years	91.4	8.7	46.5	44.9	8.7	0	127

tion on the leanness-fatness continuum operate in describing general appearance of the men in service two to seven years and over seven years, judging from the trend to larger numbers in these groups with per cent "standard weight" less than 90 (Table VII).

BIOCHEMICAL STUDIES

The biochemical studies consisted of certain analyses of fasting blood and urine samples collected from every fourth man given a physical examination. To facilitate collection and assure fasting, the men in the biochemistry sample were quartered in the same barracks for the night. The six-hour fasting urine collection was started at 12 midnight and concluded at 6 A.M. Blood samples were drawn as soon as possible after the urine collection period. Determinations of plasma protein, hemoglobin (copper sulfate-specific gravity method) and hematocrit (by centrifugation) were completed in the field. Plasma samples were refrigerated and shipped by airplane to the base laboratory in Tehran where they were analyzed for vitamin A, carotene, and vitamin C content. To stabilize vitamin C against oxidation, metaphosphoric acid-filtrates were prepared in the field. Urine samples were analyzed for thiamine, riboflavin, N′-methylnicotinamide (as the urinary excretion product of niacin), and creatinine.

The number of men examined biochemically were: 126 in Tehran, seventy-two in Ahwaz, seventy-nine in Mashad, sixty-six in Mahabad, seventy-two in Rasht and seventy-three in Khash.

Methods

Fifteen milliliter centrifuge tubes were prepared containing either heparin or the dried oxalate mixture of Heller and Paul[15] sufficient to prevent coagulation of 20 ml. of blood. Ten milliliter vaccine vials containing dried anticoagulant were used to receive the 2 ml. of whole blood for determination of hemoglobin and hematocrit. As soon as the plasma was separated, 4 ml. of plasma was placed in 22 ml. screwcap vials containing 12 ml. of 6 per cent metaphosphoric acid (HPO_3) for the "vitamin C filtrate" samples.

Urine volumes were recorded and 50 ml. aliquots were placed in screwcap bottles con-

TABLE XI
Hematocrit Determinations with Centrifugation on Clinical Table Model and Orbit-Floor Model Centrifuge

Sample No.	Clinical Model Centrifuge		Orbit-Floor Model Centrifuge		Clinical 45 min. vs. Orbit 30 min.
	30 min.	45 min.	30 min.	45 min.	
A	48.7	47.9	48.0	47.9	−0.1
B	52.8	52.6	51.1	50.9	+1.5
C	52.5	52.0	51.5	51.4	+0.5
D	47.6	47.4	47.0	45.7	+0.4

taining 100 mg. of oxalic acid. All urine samples were acidulated with hydrochloric acid for preservation of vitamins.

The blood samples were drawn before the men were permitted to have breakfast. Preparation of the plasma was begun as soon as the samples were cooled. At this time, the determinations of hemoglobin and hematocrit on the whole blood were started. Usually two hours were required for the drawing of seventy blood samples and six to eight hours more for the preparation of plasma, determination of total plasma proteins, preparation of the vitamin C filtrates, and centrifugation of the hematocrit tubes.

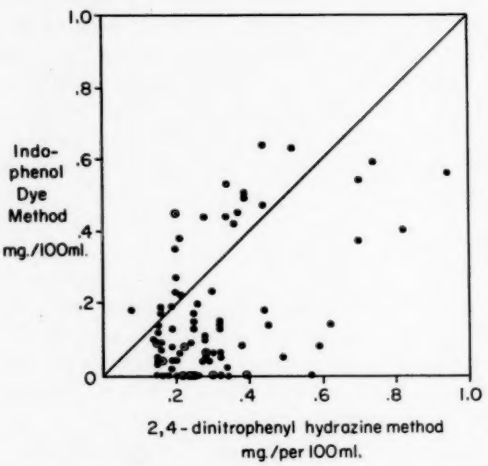

FIG. 3. Average serum vitamin C levels determined by two methods on ninety-one samples. Method A: 2,4-dinitrophenylhydrazine, 0.296 mg. per 100 ml. Method B: Indophenol dye, 0.155 mg. per 100 ml. Difference between method A and method B, 0.141. Standard deviation of differences, 0.170. Standard error of average difference 0.0178. $t = 7.9$. Probability level <0.001.

The urine aliquots, plasma and vitamin C filtrates were kept cold in Marmite refrigerator cans during shipment. As soon as the samples reached the main laboratory, they were transferred to a deep freeze except for the urine aliquots which were stored in the refrigerator. Usually all the analyses for seventy samples could be completed in three working days.

The analytic methods employed were standard procedures selected for their simplicity and accuracy. Since certain of the analyses were performed "in the field," rapid methods for the determination of hemoglobin and total plasma proteins were employed. These were the copper sulfate-specific gravity methods described by Phillips and Van Slyke et al.[16–18] as set forth in "Metabolic Methods."[19] The per cent packed red cells (hematocrit) was determined by the Wintrobe method[20] after centrifugation of the tubes at top speed (3,000 r.p.m.) in a table model clinical centrifuge for forty-five minutes. This method was selected after comparing duplicate determinations from a floor model "Orbit" centrifuge equipped with a tachometer with the results obtained from the clinical model (Table XI). In some of the Army hospitals the current was considerably less than 220 volts, which necessitated centrifugation for longer than forty-five minutes.

Plasma vitamin A and carotene levels were determined by the Carr-Price method with antimony trichloride.[21]

The vitamin C concentration in the plasma was determined on some of the initial samples

TABLE XII
Plasma Protein, Hemoglobin and Hematocrit Levels by Location

	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Total
No. of troops	122	72	79	66	70	71	480
Plasma protein (gm./100 ml.)							
<6.0	0	0	0	0	0	0	(0)
6.0–6.4	0	0	0	0	1.4	1.4	(0.4)
6.5–7.0	1.6	0	0	0	12.9	2.8	(2.7)
>7.0	98.4	100.0	100.0	100.0	85.7	95.8	(96.9)
No. of troops	122	71	79	63	66	71	472
Hemoglobin (gm./100 ml.)							
<12.0	0.8	1.4	3.8	1.6	0	2.8	(1.7)
12.0–13.9	7.4	38.0	15.2	28.6	12.1	28.2	(19.9)
14.0–15.0	16.4	26.8	10.1	30.2	19.7	12.7	(18.6)
>15.0	75.4	33.8	70.9	39.6	68.2	56.3	(59.8)
No. of troops	123	68	78	63	64	73	469
Hematocrit (per cent)							
<36	0.8	5.9	3.8	6.3	1.6	2.7	(3.2)
36–41	1.6	8.8	0	4.8	0	5.5	(3.2)
42–45	13.8	39.7	10.3	14.3	12.5	21.9	(18.1)
>45	83.8	45.6	85.9	74.6	85.9	69.9	(75.5)

NOTE: Bold face figures represent per cent of troops; figures in parentheses represent averages.

using the indophenol "15-second-reading" method.[22–24] The distilled water used was demineralized to free it of copper ions (Cu^{++}). Even so, inconsistent results were obtained by this method. Many of the plasma and filtrate samples were analyzed for vitamin C by the 2,4-dinitrophenylhydrazine method of Roe and Keuther[25] as modified by Schaffert and Kingsley.[26] A comparison of the values obtained by the two methods is given in Figure 3. Ascorbic acid levels were determined by the indophenol method on fresh plasma and comparative analyses were made after the samples or filtrates had been stored in the deep freeze for about ten days. The phenylhydrazine method gave higher and more consistent results; therefore, the Roe-Keuther method was used to determine ascorbic acid concentration in all subsequent plasma filtrate samples. In some cases, insufficient plasma was obtained to permit use of more than 2 or 3 ml. for the vitamin C filtrate and still leave a sufficient sample for the vitamin A and carotene determinations; when this was the case, the filtrate was diluted to 16 ml. final volume with HPO_3 prior to analysis.

Riboflavin levels in the urine were determined by the method of Conner and Straub,[27] while thiamine was measured by the thiochrome procedure as described by Emmett et al.,[28] Mason and Williams,[29] and Perlzweig and coworkers.[30] N′-methylnicotinamide was determined by the method of Huff, Perlzweig and Tilden.[31]

Some difficulty was encountered with the urine blanks and internal standards in all of these fluorometric methods so that it became necessary to assign limits within which the internal standard values were used in the calculations. In those cases in which the internal standard values fell outside the assigned limits, the fluorescence of the standard was taken from the "external" standard curve. Creatinine was measured by the picric acid method of Folin and Wu.[32]

Blood and urine samples reached the main laboratory promptly from the surveys carried out in the city of Tehran. However, because

TABLE XIIIA
Plasma Vitamin C, Vitamin A and Carotene Levels by Location

	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Total
No. of troops	121	71	78	63	69	67	469
Plasma vitamin C (mg./100 ml.)							
<0.10	**37.2**	**31.0**	**19.2**	**44.4**	**24.6**	**94.0**	(40.5)
0.10–0.19	**23.1**	**12.7**	**10.3**	**28.6**	**23.2**	**4.5**	(17.5)
0.20–0.40	**28.1**	**45.1**	**48.7**	**22.2**	**29.0**	**1.5**	(29.6)
>0.40	**11.6**	**11.2**	**21.8**	**4.8**	**23.2**	**0**	(12.4)
No. of troops	117	70	78	58	66	72	461
Plasma vitamin A (μg./100 ml.)							
<10	**1.7**	**0**	**6.4**	**19.0**	**1.5**	**18.1**	(6.9)
10–19	**8.5**	**20.0**	**12.8**	**31.0**	**33.3**	**50.0**	(23.9)
20–50	**86.4**	**75.7**	**78.2**	**50.0**	**62.1**	**31.9**	(66.8)
>50	**3.4**	**4.3**	**2.6**	**0**	**3.1**	**0**	(2.4)
No. of troops	118	70	78	64	69	72	471
Plasma carotene (μg./100 ml.)							
<20	**0**	**0**	**1.3**	**17.2**	**5.8**	**48.6**	(10.8)
20–39	**18.6**	**75.7**	**60.2**	**76.6**	**65.2**	**47.2**	(53.1)
40–100	**81.4**	**24.3**	**38.5**	**6.2**	**29.0**	**4.2**	(36.1)
>100	**0**	**0**	**0**	**0**	**0**	**0**	(0)

NOTE: Bold face figures represent per cent of troop; figures in parentheses represent averages.

TABLE XIIIB
Plasma Vitamin C, Vitamin A and Carotene Levels by Location (Per Cent of Troops)

	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Average
Plasma vitamin C (mg./100 ml.)							
0–0.19	60.3	43.7	29.5	73.0	47.8	98.5	58.0
>0.19	39.7	56.3	70.5	27.0	52.2	1.5	42.0
Plasma vitamin A (mg./100 ml.)							
0–19	10.2	20.0	19.2	50.0	34.8	68.1	30.8
>19	89.8	80.0	80.8	50.0	65.2	31.9	69.2
Plasma carotene (mg./100 ml.)							
0–39	18.6	75.7	61.5	93.8	71.0	95.8	63.9
>39	81.4	24.3	38.5	6.2	29.0	4.2	36.1

of transportation difficulties there were delays in returning the samples during the field trips. During these delays, the plasma and vitamin C filtrates were kept in the cold (0 to 4° c.).

Results

The results of the biochemical analyses are given in Tables XII, XIII and XIV by location, in Tables XV and XVI by type of unit, and in Tables XVII and XVIII by time in service. These results are expressed in accordance with standards proposed by ICNND, as given in Table XIX.

Plasma protein, hemoglobin, and hematocrit data (Table XII) reveal no appreciable differences among the six locations. In general, these tests indicate that protein intake was sufficient, and no severe anemia problem exists.

TABLE XIVA
Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Location

	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Total
No. of troops	113	72	75	65	71	73	469
Thiamine (μg./6 hr.)							
<10	**7.1**	**0**	**0**	**0**	**0**	**0**	**(1.7)**
10–24	**17.7**	**4.2**	**0**	**0**	**4.2**	**4.1**	**(6.2)**
25–50	**39.8**	**20.8**	**0**	**0**	**9.9**	**4.1**	**(14.9)**
>50	**35.4**	**75.0**	**100.0**	**100.0**	**85.9**	**91.8**	**(77.2)**
No. of troops	115	73	75	65	70	73	471
Riboflavin (μg./6 hr.)							
<10	**17.4**	**4.1**	**18.7**	**1.5**	**1.4**	**2.7**	**(8.7)**
10–29	**7.8**	**26.0**	**12.0**	**3.0**	**10.0**	**20.5**	**(12.9)**
30–100	**62.6**	**53.4**	**45.3**	**69.3**	**68.6**	**53.4**	**(58.9)**
>100	**12.2**	**16.5**	**24.0**	**26.2**	**20.0**	**23.3**	**(19.5)**
No. of troops	114	71	75	65	70	73	468
N′-Methylnicotinamide (mg./6 hr.)							
<0.20	**0.9**	**1.4**	**0**	**0**	**0**	**0**	**(0.4)**
0.20–0.59	**2.6**	**1.4**	**0**	**1.5**	**1.4**	**0**	**(1.3)**
1.60–1.60	**55.3**	**46.5**	**28.0**	**58.5**	**17.1**	**8.2**	**(37.0)**
>1.60	**41.2**	**50.7**	**72.0**	**40.0**	**81.5**	**91.8**	**(61.3)**

NOTE: Bold face figures represent per cent of troops; figures in parentheses represent averages.

TABLE XIVB
Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Location (Per Cent of Troops)

	Tehran	Ahwaz	Mashad	Mahabad	Rasht	Khash	Average
Thiamine (μg./6 hr.)							
0–24	24.8	4.2	0	0	4.2	4.1	7.9
>24	75.2	95.8	100	100	95.8	95.9	92.1
Riboflavin (μg./6 hr.)							
0–29	25.2	30.1	30.7	4.5	11.4	23.2	21.6
>29	74.8	69.9	69.3	95.5	88.6	76.7	78.4
N′-Methylnicotinamide (mg./6 hr.)							
0-0.59	3.5	2.8	0	1.5	1.4	0	1.7
>0.59	96.5	97.2	100	98.5	98.6	100	98.3

However, a slightly low hemoglobin concentration was noted in many subjects. The following percentages of the troops had concentrations of hemoglobin less than 14 gm. per 100 ml. of blood: Ahwaz, 39 per cent; Khash, 31 per cent; Mahabad, 30 per cent; Mashad, 19 per cent; Rasht, 12 per cent, and Tehran, 8 per cent. Values below 14 gm. per 100 ml. of plasma are considered to be below average for young adult males living at sea level.

Plasma vitamin C, vitamin A and carotene values are given by location in Table XIII. Approximately 50 per cent of all the troops had "low" or "deficient" vitamin C plasma values by the standards of the ICNND Manual, 1957 edition,[13] i.e., less than 0.2 mg. per cent. The highest prevalence of "deficient" values,

TABLE XVA
Plasma Vitamin C, Vitamin A and Carotene Levels by Unit

	Infantry	Infantry Training	Artillery	Military Police	Engineers	Cavalry	Medical
No. of troops	195	104	66	28	23	27	26
Plasma vitamin C (mg./100 ml.)							
<0.10	**45.1**	**42.3**	**36.4**	**46.4**	**30.4**	**0**	**53.9**
0.10–0.19	**20.0**	**17.3**	**12.1**	**28.6**	**21.8**	**0**	**15.4**
0.20–0.40	**24.6**	**28.9**	**34.8**	**17.8**	**47.8**	**55.6**	**26.9**
>0.40	**10.3**	**11.5**	**16.7**	**7.2**	**0**	**44.4**	**3.8**
No. of troops	195	103	67	28	18	25	25
Plasma vitamin A (μg./100 ml.)							
<10	**8.7**	**2.9**	**1.5**	**0**	**27.8**	**0**	**24.0**
10–19	**33.3**	**14.6**	**19.4**	**17.9**	**22.2**	**0**	**32.0**
20–50	**55.4**	**79.6**	**77.6**	**78.6**	**50.0**	**96.0**	**44.0**
>50	**2.6**	**2.9**	**1.5**	**3.5**	**0**	**4.0**	**0**
No. of troops	198	104	68	28	23	25	25
Plasma carotene (μg./100 ml.)							
<20	**16.2**	**4.8**	**1.5**	**0**	**21.7**	**0**	**32.0**
20–39	**54.0**	**55.8**	**63.2**	**3.6**	**73.9**	**36.0**	**60.0**
40–100	**29.8**	**39.4**	**35.3**	**96.4**	**4.4**	**64.0**	**8.0**
>100	**0**	**0**	**0**	**0**	**0**	**0**	**0**

NOTE: Bold face figures represent per cent of troops; figures in parentheses represent averages.

TABLE XVB
Plasma Vitamin C, Vitamin A and Carotene by Unit (Per Cent of Troops)

	Infantry	Infantry Training	Artillery	Military Police	Engineers	Cavalry	Medical
Plasma vitamin C (mg./100 ml.)							
0–0.19	65.1	59.6	48.5	75.0	52.2	0	69.3
>0.19	34.0	40.4	51.5	25.0	47.8	100	30.7
Plasma vitamin A (μg./100 ml.)							
0–19	32.0	17.5	20.9	17.9	50.0	0	56.0
>19	58.0	82.5	79.1	82.1	50.0	100	44.0
Plasma carotene (μg./100 ml.)							
0–39	70.2	60.6	64.7	3.6	95.6	36	92.0
>39	29.8	39.4	35.3	96.4	4.4	64	8

i.e., less than 0.1 mg. per cent, were noted in the troops at Khash (94 per cent), Mahabad (44 per cent), and Tehran (37 per cent), whereas at Mashad only 19 per cent had these "deficient" values. Even with these low values no frank scurvy was seen. The greatest prevalence of "deficient" or "low" plasma values of vitamin A and carotene was found in troops in the Mahabad and Khash areas. Vitamin A levels were "deficient" (10 μg. per cent or less) in 19 per cent of those examined in Mahabad and in 18 per cent in Khash. In addition, 31 per cent of the troops in Mahabad and 50

TABLE XVIA

Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Unit

	Infantry	Infantry Training	Artillery	Military Police	Engineers	Cavalry	Medical
No. of troops	191	107	66	27	25	26	27
Thiamine (μg./6 hr.)							
<10	**0**	**1.9**	**0**	**18.6**	**0**	**3.8**	**0**
10–24	**1.6**	**8.4**	**1.5**	**14.8**	**0**	**34.6**	**11.1**
25–50	**11.5**	**25.2**	**4.5**	**33.3**	**0**	**27.0**	**7.4**
>50	**86.9**	**64.5**	**94.0**	**33.3**	**100.0**	**34.6**	**81.5**
No. of troops	190	108	66	29	25	26	27
Riboflavin (μg/6 hr.)							
<10	**4.7**	**11.1**	**9.1**	**10.3**	**0**	**34.6**	**7.4**
10–29	**14.2**	**15.8**	**6.0**	**3.4**	**8.0**	**7.7**	**29.6**
30–100	**62.1**	**50.0**	**62.1**	**72.4**	**68.0**	**53.8**	**44.5**
>100	**19.0**	**23.1**	**22.7**	**13.9**	**24.0**	**3.9**	**18.5**
No. of troops	190	107	66	28	25	26	26
N′-Methylnicotinamide (mg./6 hr.)							
<0.20	**0**	**0.9**	**0**	**0**	**0**	**3.8**	**0**
0.20–0.59	**1.0**	**2.0**	**0**	**0**	**4.0**	**3.8**	**0**
0.60–1.60	**33.2**	**34.5**	**33.3**	**64.3**	**56.0**	**57.7**	**15.4**
>1.60	**65.8**	**62.6**	**66.7**	**35.7**	**40.0**	**34.5**	**84.6**

NOTE: Bold face figures represent per cent of troops; figures in parentheses represent averages.

TABLE XVIB

Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Unit (Per Cent of Troops)

	Infantry	Infantry Training	Artillery	Military Police	Engineers	Cavalry	Medical
Thiamine (μg./6 hr.)							
0–24	1.6	10.3	1.5	33.4	0	38.4	11.1
>24	98.4	89.7	98.5	66.6	100	61.6	88.9
Riboflavin (μg./6 hr.)							
0–29	18.9	26.9	15.1	13.7	8.0	42.3	27.0
>29	81.1	73.1	84.8	86.3	92.0	57.7	63.0
N′-Methylnicotinamide (mg./6 hr.)							
0–0.59	1	2.9	0	0	4.0	7.6	0
>0.59	99	97.1	100	100	96.0	92.2	100

per cent of those in Khash had "low" (11 to 19 μg. per cent) plasma vitamin A levels. In 17 per cent of the troops in Mahabad and 49 per cent of those in Khash the carotene levels were less than 20 μg. per cent (deficient), in 77 per cent of the troops in Mahabad and 47 per cent in Khash, carotene levels were "low" (20 to 39 μg. per cent). The highest values for vitamin A and carotene were found in the troops in Tehran; 90 per cent had "acceptable" vitamin A levels (20 μg. per cent or more) and 81 per cent had "acceptable" carotene values (40 μg. per cent or more). For all locations, approximately 30 per cent had "low" to "deficient" vitamin A levels (less than 20 μg. per cent), and 65 per cent had "low" carotene plasma values (less than 40 μg. per cent).

TABLE XVIIA
Plasma Vitamin C, Vitamin A and Carotene Levels by Time in Service

	0–6 Mo.	7–12 Mo.	13–24 Mo.	3–7 Yr.	>7 Yr.
No. of troops	149	88	171	41	20
Plasma vitamin C (mg./100 ml.)					
<0.10	**34.9**	**45.5**	**38.6**	**65.9**	**25.0**
0.10–0.19	**14.1**	**22.7**	**18.7**	**7.3**	**30.0**
0.20–0.40	**33.6**	**23.9**	**32.8**	**19.5**	**20.0**
>0.40	**17.4**	**7.9**	**9.9**	**7.3**	**25.0**
No. of troops	141	89	171	41	19
Plasma vitamin A (μg./100 ml.)					
<10	**1.4**	**11.2**	**9.4**	**7.3**	**5.3**
10–19	**16.3**	**33.7**	**30.4**	**9.8**	**5.3**
20–50	**79.4**	**55.1**	**57.9**	**78.0**	**84.2**
>50	**2.9**	**0**	**2.3**	**4.9**	**5.3**
No. of troops	147	90	174	41	19
Plasma carotene (μg./100 ml.)					
<20	**8.2**	**14.5**	**12.6**	**7.3**	**5.3**
20–39	**55.1**	**52.2**	**54.6**	**43.9**	**47.4**
40–100	**36.7**	**33.3**	**32.8**	**48.8**	**47.4**
>100	**0**	**0**	**0**	**0**	**0**

NOTE: Bold figures represent per cent of troops; figures in parentheses represent averages.

TABLE XVIIB
Plasma Vitamin C, Vitamin A and Carotene Levels by Time in Service (Per Cent of Troops)

	0–6 Mo.	7–12 Mo.	13–24 Mo.	3–7 Yr.	>7 Yr.
Plasma vitamin C (mg./100 ml.)					
0–0.19	49.0	68.2	57.3	73.2	55.0
>0.19	51.0	31.8	42.7	26.8	45.0
Plasma vitamin A (μg./100 ml.)					
0–19	17.7	44.9	39.8	17.1	10.6
>19	82.3	55.1	60.2	82.9	89.5
Plasma carotene (μg./100 ml.)					
0–39	63.3	66.7	67.2	51.2	52.7
>39	36.7	33.3	32.8	48.8	47.4

Plasma vitamins A, C and carotene values are given by time in service in Table XVIIA. By grouping "low" and "deficient" ranges together and comparing them with combined values for "acceptable" and "high" ranges for service of zero to six months and seven to twelve months as compared with thirteen to twenty-four months and three to seven years taken together, as is done in Table XVIIB, further evidence is adduced that the ration is low in these three nutrients. In fact, with regard to vitamins A and C, the troops' status deteriorates with time in service. Just over half of the recruits have "acceptable" or "high" plasma vitamin C values, but this is found to decrease to less than a third in the second half of their first year of service, although it is found to increase a little during the second year. Only a quarter of those with three through seven years military duty have "acceptable" or "high" values. In fact, the recruits are better off in this regard than any others.

TABLE XVIIIA
Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Time in Service

	0–6 Mo.	7–12 Mo.	13–24 Mo.	3–7 Yr.	>7 Yr.
No. of troops	151	88	170	41	19
Thiamine (μg./6 hr.)					
<10	**1.3**	**2.3**	**1.8**	**0**	**5.3**
10–24	**10.6**	**8.0**	**2.3**	**2.4**	**5.3**
25–50	**22.5**	**9.0**	**10.6**	**22.0**	**5.3**
>50	**65.6**	**80.7**	**85.3**	**75.6**	**84.2**
No. of troops	152	88	171	41	19
Riboflavin (μg./6 hr.)					
<10	**13.8**	**5.7**	**7.0**	**4.9**	**5.3**
10–29	**15.1**	**11.3**	**12.9**	**12.2**	**5.3**
30–100	**54.0**	**59.1**	**63.7**	**56.1**	**57.9**
>100	**17.1**	**23.9**	**16.4**	**26.8**	**31.6**
No. of troops	151	87	170	41	19
N′-Methylnicotinamide (mg./6 hr.)					
<0.20	**1.3**	**0**	**0**	**0**	**0**
0.20–0.59	**1.3**	**1.2**	**0.6**	**2.4**	**5.3**
0.60–1.60	**38.4**	**37.9**	**38.8**	**22.0**	**36.8**
>1.60	**59.0**	**60.9**	**60.6**	**75.6**	**57.9**

NOTE: Bold figures represent per cent of troops; figures in parentheses represent averages.

TABLE XVIIIB
Urinary Excretion of Thiamine, Riboflavin and N′-Methylnicotinamide by Time in Service (Per Cent of Troops)

	0–6 Mo.	7–12 Mo.	13–24 Mo.	3–7 Yr.	>7 Yr.
Thiamine (μg./6 hr.)					
0–24	11.9	10.3	4.1	2.4	10.6
>24	88.1	89.7	95.9	97.6	89.5
Riboflavin (μg./6 hr.)					
0–29	28.9	17.0	19.9	17.1	10.6
>29	71.1	83.0	80.1	82.9	89.5
N′-Methylnicotinamide (mg./6 hr.)					
0–0.59	2.6	1.2	0.6	2.4	5.3
>0.59	97.4	98.8	99.4	97.6	94.7

The situation is similar with regard to vitamin A during two years of duty, but with a larger proportion having "acceptable" or "high" serum values. Once the two years have been completed, however, and in contrast to vitamin C, the soldier is as well off, or better off, than the recruit so far as his serum vitamin A level is concerned.

Plasma carotene shows the least favorable levels of these three nutrients, with a similar trend, but once two years in service are over, chances for a good plasma carotene level are much improved.

In Tables xvA and xvB these data on plasma levels of vitamins A, C and carotene support the general impression already noted that the nutritional status of recruits with respect to these indicators is better than that of

TABLE XIX
Guide Used in Interpretation of Blood and Urine Data in Young Adult Males

	Deficient	Low	Acceptable	High
Blood Data				
Total plasma protein (gm./100 ml.)	<6.0	6.0–6.4	6.5–7.0	>7.0
Hemoglobin (gm./100 ml.)	<12.0	12.0–13.9	14.0–15.0	>15.0
Hematocrit (PCV) in per cent	<36	36–41	42–45	>45
Plasma vitamin C (mg./100 ml.)	<0.1	0.10–0.19	0.2–0.4	>0.4
Plasma vitamin A (μg./100 ml.)	<10	10–19	20–50	>50
Plasma carotene (μg./100 ml.)	<20	20–39	40–100	>100
Urine Data				
Thiamine (μg./gm. creatinine)	<27	27–65	66–130	>130
Riboflavin (μg./gm. creatinine)	<27	27–79	80–270	>270
N′-Methylnicotinamide (mg./gm. creatinine)	<0.5	0.5–1.59	1.6–4.3	>4.3

soldiers further along on their two-year tour of duty. The soldiers from the training regiments have higher values in each instance than those with longer service in the infantry regiments. Men from the cavalry all have "acceptable" or "high" levels of vitamins A and C, with artillery next at 50 per cent for vitamin C, and the military police and the infantry trainees both at 82 per cent for vitamin A. The military police also had the highest proportion of "acceptable" and "high" values for carotene.

Urinary excretion values for thiamine, riboflavin, and niacin (as N′-methylnicotinamide) by location are given in Table XIVA. "Deficient" values of thiamine excretion (9 μg. or less per six hours) were found only in the troops in Tehran (7 per cent), with an additional 18 per cent of the troops excreting only a "low" level (10 to 24 μg. per six hours). Troops in the other areas appeared to be in a satisfactory state of thiamine nutrition. All troops sampled in the Mahabad and Mashad areas had "high" thiamine excretion levels (over 50 μg. per six hours).

The prevalence of "deficient" excretion of riboflavin (9 μg. per six hours) was the highest in Tehran and Mashad (17 and 19 per cent). Of all troops sampled, approximately 21 per cent had "low" to "deficient" values (29 μg. or less per six hours) (Table XIVB).

In general, 98 per cent of the troops had "acceptable" to "high" levels (0.6 μg. or more per six hours) of N′-methylnicotinamide excretion.

Again combining "deficient" and "low" values and comparing them with the combined "acceptable" and "high" values by time in service in Table XVIIIB we find that nutriture as represented by excretion values of thiamine and riboflavin appears to improve with time in service, not only during the first two years, but also throughout several years of service in the case of riboflavin, with thiamine values decreasing to first year levels after more than seven years in service. Time appears to have no relation to the excellent levels of N′-methylnicotinamide excretion.

Combining "deficient" with "low" and "acceptable" with "high" excretion values for thiamine in Table XIVB, values for "low" levels of thiamine excretion in Tehran exceed three other areas by a factor of 6 and the remaining two areas had no values in this range. Table XVIIIB shows the high percentage of "low" values for the first year of service, 12 per cent for the first six months and 10 per cent for the second six months, with 11 per cent for those with over seven years of service, and the lowest percentage for those in the second through the seventh years.

Study of Table XVIB indicates that the greatest number of persons with "low" thiamine excretion levels are in the military police and the cavalry, with about one third of the

samples falling in this category. Medical personnel and training troops both have about 10 per cent with "low" thiamine excretion levels. The infantry troops with more than six months of service, artillerymen and engineers have only 1.6, 1.5 and zero per cent, respectively.

The cavalry also had the highest percentage of "low" excretion levels of riboflavin (42 per cent) followed by the medical group and infantry trainees (27 per cent each). The military police, however, were as well off as any unit in regard to riboflavin excretion (Table XVIA). In contrast to plasma levels of vitamins A and C, the thiamine and riboflavin status of the infantry combat troops was better than that of the recruits.

Comments

In most cases the concentration of vitamins in the blood or the amount excreted in the urine can be taken as a measure of the dietary intake of these vitamins, when compared with levels in the blood or urine of well nourished persons. Levels which are low often signal nutrient deficiencies which may later become clinically discernible, if, indeed, such signs and symptoms are not already present.

On the basis of the biochemical data, the intake of vitamin C, vitamin A, and riboflavin by the troops would seem to be inadequate to maintain tissue concentrations at a level consistent with a state of good health. Approximately 70 per cent of those examined in Khash, 50 per cent of those in Mahabad, 20 per cent of those in Ahwaz and Mashad, and 35 per cent of those in Rasht had plasma concentrations of vitamin A suggestive of inadequate intake of this vitamin. A large percentage of the subjects had plasma vitamin C levels which would place them in the category indicative of inadequate intake of the vitamin; from 30 to 70 per cent of the troops fell into this category. In Khash, 98 per cent of those examined would belong in this group. In all of the areas surveyed from 10 to 30 per cent of the men had urinary riboflavin levels which indicated a minimal intake of riboflavin. The troops at Mahabad present a possible exception to this statement.

TABLE XX
Average Percentage of Nutrients Lost During Cooking

Food	Thiamine	Riboflavin	Niacin	Vitamin C
Meats	35	20	25	..
Meats, plus drippings	25	5	10	..
Eggs	25	10	0	..
Cereals	10	0	10	..
Legumes	20	0	0	..
Vegetables, leafy, green and yellow	40	25	25	60
Tomatoes	5	5	5	15
Vegetables, other	25	15	25	60
Potatoes	40	25	25	60
Rice*	33	33	..	..
Flour*	25	15	20	..

NOTE: Reference: U. S. Department of the Army Technical Manual 8-501, Nutrition, Table II, p. 19, September 1949.
* Estimate based on findings of U. S. Army Medical Research and Nutrition Laboratory.

The two other vitamins studied by biochemical methods, niacin and thiamine, seem to have been present in the diets of the troops in adequate amounts since only 1 to 4 per cent of the samples fell into the excretion range indicative of dietary insufficiencies of these vitamins. An exception might be indicated by thiamine excretions in Tehran, where one quarter of the troops had excretions suggestive of inadequate thiamine intake.

In summary, the biochemical evidence suggests that the dietary intake of vitamin C, vitamin A, and riboflavin was less than desirable. This means that even though functional impairment may not yet be evident as a result of the inadequate intake, the subjects would probably not be adequately protected during times of stress (illness, fever, or periods of short rations). The tissue reserves of these vitamins are undoubtedly low.

DIETARY STUDIES

Types of Food

Dietary surveys were conducted in messes where approximately 1,000 troops were fed. The dietary appraisal consisted of determining the quantity of food available for consumption

TABLE XXI
Tehran Winter Master Menu (Food Allowances in Grams per Man per Day)

Food	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Total
Rice, 1st class	175	175	175	...	175	175	...	875
Rice, 2nd class	...	75	...	75	...	75	75	300
Vegetable oil	30	25	20	5	20	25	7	132
Peas	15	20	20	20	20	20	20	135
White beans	...	30	30	30	30	30	30	180
Black-eyed peas	35	...	...	...	...	...	...	35
Lentils	75	...	...	...	...	...	...	75
Split peas	...	20	...	...	20	...	30	70
Dry lemon	2	2	6	...	4	4	2	20
Condiment	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.5
Parsley	25	...	...	20	...	...	...	45
Beets	...	20	...	20	...	20	...	60
Spinach	25	20	75	...	...	95	75	290
Leek	25	20	...	20	...	20	...	85
Coriander	...	15	...	15	...	15	...	45
Onion	25	40	45	70	45	40	45	310
Potatoes	...	25	50	100	75	...	50	300
Mutton	125	175	225	225	225	175	125	1,275
Salt	20	35	25	30	35	35	30	210
Plums	...	...	...	...	...	...	30	30
Carrots	...	...	...	30	...	...	...	30
Sugar	...	...	...	...	...	...	...	250*
Tea	...	...	...	...	...	...	...	12.5†
Bread	1,000	1,000	1,000	1,000	1,000	1,000	1,000	7,000‡
Orange	...	...	...	...	50	...	...	50§

* Sugar is issued at rate of 1 kg. per month.
† Tea is issued at rate of 50 gm. per month.
‡ Bread is issued once each twenty-four hours and the men consume it as they desire.
§ This item was an extra issue and was not part of the master menu.

per man per day during a five-day period. A record was made of the food issued to the mess, and the number of men being fed. Losses in preparation and food waste were estimated. From these data, amounts of the various foods consumed per man per day were calculated in grams. The nutrients provided per man per day were then calculated by means of the values contained in references given in the bibliography.[33–36] The nutritive evaluations of the rations were corrected for cooking losses (Table xx).

The food offered members of the Imperial Iranian Army is based upon master summer and winter menus made up twice a year in Tehran. During the time the survey was made the winter menu was in use. These menus are used only as guides, and may be modified to meet local preferences and local supplies. If any change is made the Quartermaster, the Surgeon and the Commanding Officer must concur. The winter master menu is shown in Table xxi.

Nonperishable items are stored in a central depot. A six-month supply is kept on hand. The stock is rotated so no food is stored more than six months. In every case, the storerooms were in excellent condition with all foods raised from the floors on pallets. These foods are usually furnished by a local contractor and are carefully inspected at time of delivery.

Perishable foods, which include meat, fresh vegetables and fruits, are also supplied by a local contractor and are usually delivered daily since refrigeration is not available.

Food is issued daily from the central stores and the issue is based upon the troop strength report. The various foods are carefully weighed in the presence of a Quartermaster officer,

the mess personnel, and the officer of the day. Occasionally a forty-eight-hour issue of food may be made, but this is unusual.

The food is transported to kitchens which are usually designed to feed 1,000 to 1,200 men. The principal cooking utensils are large copper pots set in concrete and fired from a stove located outside the kitchen. Fuel oil is the usual source of heat, as Iran is a petroleum-rich country. These pots are tinned so that the food does not come in contact with the copper. The belief is prevalent that if the pots are not tinned and the food touches the copper, the food will be made toxic. The Iranians were very definite on this point. It is said that all such pots are retinned every fifteen days.

Vegetables are carefully prepared with a minimum of waste. They are placed in the large copper pot along with the other foods for the preparation of a stew called *aschi*.

Typical food for one day is as follows:

Breakfast	*Lunch*	*Supper*
white beans	mutton	potatoes
pumpkin	spinach	onions
carrots	beets	rice
vegetable oil	beans	mutton
onions	peas	vegetable oil
	rice	split peas
	onions	dried lemon
	vegetable oil	condiments
	pepper	salt
	yellow wood	
	salt	

There are no mess halls, so it is necessary for each organization to bring large containers to the main kitchen where a measured amount of food is issued, the quantity depending upon the troop strength report. This food is taken to the barracks where an issue is made to the individual. The prepared food was always completely consumed.

Bread is issued once a day. The bread issue for all places was 1 kg. per man per day, except at Rasht. The commanding officer in Rasht requested and was allowed a lesser bread allowance to permit an issue of rice. Rasht is situated among the rice fields bordering the Caspian Sea where the men are accustomed to rice as a diet staple.

The ovens were operated to capacity twenty-four hours a day baking the Iranian type of bread. Even so, they could not bake enough bread to supply the troops so an experimental type of bread which resembled the typical American loaf was baked in Tehran. Adequate time was not allowed to bake the interior of the loaves. This plus the absence of yeast resulted in a "half-baked" loaf with a soggy interior which the men did not like and so the soggy portion of the loaf was usually discarded. Since the bread was issued once a day and each man consumed the bread as he wished, and not necessarily at mealtime, it was difficult to estimate the actual consumption of this latter type of bread. Where the indigenous, flat-type of bread was furnished, there was no evidence of its being thrown away.

There was some variation in the type of flour issued at the different sites surveyed. Generally speaking, the more highly refined flour was found in Tehran.

The men were also issued 1 kg. of sugar each month and 50 gm. of tea. Usually the man prepared the tea himself, but occasionally it was made in the central kitchen. Field-type rations were not available for troops on maneuvers.

Ration Survey

The same basic survey procedure was followed in each place. The current weekly menu was obtained and the issue of foodstuffs was checked. The issue was based upon troop strength for the day. Mess personnel then took this food to the kitchens. This procedure was followed for at least three days and longer when possible.

It was difficult to learn the English equivalent of the Farsi name for some of the vegetables. Examples are kadoo which meant pumpkin, and laboo which were beets.

There is a lack of adequate data on composition of foods encountered in the Near East. There is no information on cooking losses for Iranian cooking. Therefore U. S. Army percentage losses were used in the calculations (Table xx).

The detailed food and nutrient intake by areas of survey are given in Table xxii. A summary of the nutrient intake per man

TABLE XXII

Estimated Average Nutrient Intake per Man per Day
(Corrected for Conservative Vitamin Losses in Cooking*)

Food	Per Day (gm.)	Calories	Protein (gm.)	Fat (gm.)	Calcium (mg.)	Iron (mg.)	Vitamin A (I.U.)	Thiamine (mg.)	Riboflavin (mg.)	Niacin (mg.)	Vitamin C (mg.)
Tehran, Iran											
Rice	168	603	11.9	1.8	23.5	1.7	...	0.18	0.05	4.2	...
Vegetable oil	19	168	...	19.0	...	...	...	...	...	...	...
White beans	26	88	5.6	0.4	42.4	1.8	...	0.14	0.06	0.6	0.5
Dried peas	19	66	4.3	0.3	12.2	0.9	19	0.11	0.03	0.5	0.8
Black-eyed peas	50	171	11.7	0.9	38.0	2.9	20	0.37	0.09	1.0	1.0
Lentils	11	38	2.7	0.2	6.2	0.7	11	0.04	0.02	0.2	0.3
Split peas	10	34	2.5	0.1	3.3	0.5	37	0.06	0.02	0.2	0.1
Parsley	6	3	0.2	0.1	11.6	0.3	494	...	0.01	0.1	4.6
Beets	9	3	0.1	...	1.8	0.1	1	...	...	...	0.3
Spinach	41	7	0.7	0.1	27.1	1.0	3,128	0.02	0.05	0.2	7.9
Leek	12	2	0.1	...	4.6	0.1	2	...	...	...	0.4
Coriander	6	2	0.1	...	9.1	0.3	321	...	...	...	1.8
Onion	44	16	0.6	0.1	13.2	0.2	22	0.01	0.01	0.1	1.4
Potatoes	43	30	0.7	...	3.0	0.3	...	0.02	0.01	0.4	1.4
Mutton	182	439	21.7	38.4	12.7	2.5	...	0.15	0.24	5.9	...
Plums	4	2	...	...	0.6	...	13	...	...	...	0.2
Carrots	43	16	0.4	0.1	13.3	0.3	6,375	0.02	0.01	0.2	1.0
Sugar	33	128	...	...	...	...	...	...	...	...	...
Flour	536	1,876	71.8	7.5	128.6	12.9	...	1.37	0.36	8.6	...
Orange	7	2	...	...	1.7	...	8	...	...	...	2.5
Total		3,694	135.1	69.0	352.9	26.5	10,451	2.49	0.96	22.2	24.2
Ahwaz, Iran											
Rice	203	729	14.4	2.2	28.4	2.0	...	0.22	0.05	5.1	...
Vegetable oil	25	221	...	25.0	...	...	...	...	...	...	...
White beans	22	74	4.7	0.4	35.9	1.5	...	0.12	0.05	0.5	0.4
Dried peas	22	76	5.0	0.4	14.1	1.1	22	0.13	0.03	0.5	0.9
Black-eyed peas	13	44	3.0	0.2	9.9	7.4	5	0.10	0.04	0.4	0.3
Lentils	14	48	3.4	0.3	7.8	0.9	14	0.06	0.03	0.3	0.4
Split peas	6	21	1.5	0.1	2.0	0.3	22	0.03	0.01	0.1	...
Parsley	6	3	0.2	0.1	11.6	0.3	494	...	0.01	0.1	4.6
Spinach	42	7	0.8	0.1	27.7	1.0	3,205	0.02	0.05	0.2	8.1
Leek	4	1	...	...	1.5	...	1	...	...	...	0.1
Onion	44	16	0.6	0.1	13.2	0.2	22	0.01	0.01	0.1	1.4
Potatoes	51	36	0.9	0.1	3.6	0.3	...	0.03	0.01	0.5	1.6
Mutton	140	337	16.7	29.5	9.8	2.0	...	0.12	0.19	4.5	...
Dates and raisins	36	92	0.9	0.2	26.3	1.0	25	0.04	0.04	0.5	...
Sugar	33	128	...	...	...	...	...	...	...	...	...
Flour	536	1,780	74.0	10.7	198.3	22.0	...	1.81	0.59	23.2	...
Cheese	5	15	0.9	1.2	8.1	...	50	...	0.02	...	...
Milk	64	65	2.6	4.8	102.4	0.1	83	0.03	0.08	0.1	0.6
Total		3,693	129.6	75.4	500.6	40.1	3,943	2.72	1.21	36.1	18.4

* See Table XX.

Cont'd on p. 505.

per day for all areas is given in Table XXIII.

The interpretation of levels of nutrient intake is based upon the classification of "deficient," "low," "acceptable," and "high" in the ICNND standards for nutrient intake as they appear in Table XXIV. These data indicate that calcium intake is low in Tehran when any contribution from water and salt is disregarded (Table XXII). The intake of riboflavin at Tehran was the lowest found in

TABLE XXII *Continued*

Estimated Average Nutrient Intake per Man per Day
(Corrected for Conservative Vitamin Losses in Cooking*)

Food	Per Day (gm.)	Calories	Protein (gm.)	Fat (gm.)	Calcium (mg.)	Iron (mg.)	Vitamin A (I.U.)	Thiamine (mg.)	Riboflavin (mg.)	Niacin (mg.)	Vitamin C (mg.)
Mashad, Iran											
Rice	196	704	13.9	2.2	27.4	2.0	...	0.21	0.05	4.9	...
Vegetable oil	31	274	...	31.0	...	...	...	...	...	...	...
White beans	74	250	15.8	1.2	120.6	5.1	...	0.40	0.17	1.6	1.5
Dried peas	26	90	5.9	0.5	16.7	1.2	26	0.15	0.04	0.6	1.0
Black-eyed peas	37	127	8.7	0.7	28.1	2.1	15	0.27	0.07	0.07	0.7
Lentils	14	48	3.4	0.3	7.8	0.9	14	0.06	0.03	0.3	0.4
Split peas	4	14	1.0	...	1.3	0.2	15	0.02	0.01	0.1	...
Beets	86	28	1.1	0.1	17.2	0.6	13	0.01	0.03	0.2	2.4
Spinach	57	10	1.0	0.1	37.6	1.4	4,349	0.03	0.07	0.2	10.9
Turnips	107	28	1.0	0.1	36.4	0.6	11	0.04	0.04	0.6	12.0
Onion	61	23	0.8	0.1	18.3	0.3	31	0.01	0.02	0.1	2.0
Potatoes	57	40	1.0	0.1	4.0	0.3	...	0.03	0.01	0.5	1.8
Mutton	116	280	13.8	24.5	8.1	1.6	...	0.10	0.15	3.8	...
Dates and raisins	29	74	0.7	0.2	21.2	0.8	20	0.03	0.03	0.4	...
Carrots	157	58	1.6	0.3	48.7	1.1	9,625	0.06	0.05	0.7	3.8
Sugar	33	128	...	...	...	...	...	...	...	...	...
Flour	536	1,780	74.0	10.7	198.3	22.0	...	1.81	0.59	23.2	...
Milk	28	28	1.1	2.1	44.8	0.1	36	0.01	0.03	...	0.3
Catsup	15	15	0.3	0.1	1.8	0.1	282	0.01	0.01	0.3	1.7
Lemon juice	4	1	...	...	0.6	...	...	...	...	...	2.0
Grape jam	26	72	0.1	0.1	3.1	0.1	3	0.01	0.01	0.1	1.6
Total		4,072	145.2	74.4	642.0	40.5	14,420	3.26	1.41	38.3	42.1
Mahabad, Iran											
Rice	184	661	13.1	2.0	25.8	1.8	...	0.20	0.05	4.6	...
Vegetable oil	29	256	...	29.0	...	...	...	...	...	...	...
White beans	23	78	5.0	0.4	37.5	1.6	...	0.12	0.05	0.5	0.5
Dried peas	51	176	11.5	0.9	32.6	2.4	51	0.29	0.08	1.2	2.0
Black-eyed peas	21	72	5.0	0.4	16.0	1.2	8	0.15	0.04	0.4	0.4
Split peas	21	72	5.1	0.2	6.9	1.1	78	0.12	0.05	0.5	0.2
Onion	66	24	0.9	0.1	19.8	0.3	33	0.01	0.02	0.1	2.1
Potatoes	75	53	1.3	0.1	5.3	0.5	...	0.04	0.02	0.7	2.4
Mutton	177	427	21.1	37.3	12.4	2.5	...	0.15	0.24	5.7	...
Dates and raisins	36	92	0.9	0.2	26.3	1.0	25	0.04	0.04	0.5	...
Sugar	33	128	...	...	...	...	...	...	...	...	...
Flour	571	1,896	78.8	11.4	211.3	23.4	...	1.93	0.63	24.7	...
Catsup	11	11	0.2	...	1.3	0.1	207	0.01	0.01	0.2	1.2
Grape jam	26	72	0.1	0.1	3.1	0.1	3	0.01	0.01	0.1	1.6
Total		4,018	143.0	82.1	398.3	36.0	405	3.07	1.24	39.2	10.4

* See Table XX.

Cont'd on p. 506.

any area of Iran. Contributing factors were that the flour used in Tehran is more refined and the total intake of bread was less than at the other sites due to poor baking practices. Also, as previously discussed, the men preferred their traditional thin bread to loaf bread. The other nutrient which might be considered minimally available is vitamin C. Vitamin A intake, on the other hand, was excellent in Tehran, 10,451 I.U. per day, in the "high" range. Only in Mashad was a higher vitamin A intake noted.

The food intake at Ahwaz is given in Table XXII. There are slight differences from the data for Tehran. Calcium intake is 40 per cent higher than in Tehran, placing Ahwaz in

TABLE XXII *Continued*

Estimated Average Nutrient Intake per Man per Day
(Corrected for Conservative Vitamin Losses in Cooking*)

Food	Per Day (gm.)	Calories	Protein (gm.)	Fat (gm.)	Calcium (mg.)	Iron (mg.)	Vitamin A (I U.)	Thiamine (mg.)	Riboflavin (mg.)	Niacin (mg.)	Vitamin C (mg.)
Rasht, Iran											
Rice	296	1,063	21.0	3.3	41.4	3.0	...	0.32	0.08	7.4	...
Vegetable oil	30	265	...	30.1	...	...	...	...	...	...	...
White beans	38	128	8.1	0.6	61.9	2.6	...	0.20	0.09	0.8	0.8
Dried peas	29	100	6.5	0.5	18.6	1.4	29	0.17	0.04	0.7	1.2
Black-eyed peas	17	58	4.0	0.3	12.9	1.0	7	0.13	0.03	0.3	0.3
Lentils	32	111	7.7	0.6	17.9	2.0	32	0.13	0.07	0.6	1.0
Split peas	6	21	1.5	0.1	2.0	0.3	22	0.03	0.01	0.1	...
Spinach	12	2	0.2	...	7.9	0.3	916	0.01	0.01	...	2.3
Leek	12	2	0.1	...	4.6	0.1	2	...	...	...	0.4
Coriander	12	4	...	...	18.2	0.1	642	0.01	0.01	0.1	6.8
Onion	41	15	0.5	0.1	12.3	0.2	21	0.01	0.01	0.1	1.3
Potatoes	82	57	1.4	0.1	5.7	0.5	...	0.04	0.02	0.7	2.6
Mutton	116	280	13.8	24.5	8.1	1.6	...	0.10	0.15	3.8	...
Carrots	13	5	0.1	...	4.0	0.1	1,625	...	...	0.1	0.3
Sugar	33	128	...	...	...	...	...	...	...	...	...
Flour	482	1,600	66.5	9.6	178.3	19.8	...	1.63	0.53	20.8	...
Fish	21	13	1.8	0.6	3.2	0.1	4	...	0.01	0.2	...
Total		3,852	133.2	70.4	397.0	33.1	3.300	2.78	1.06	35.7	17.0

* See Table xx.

the "acceptable" range for calcium intake. This is due in part to a modest milk allowance and to the use of less refined flour. Vitamin A intake is lower, but still "acceptable." Riboflavin is "acceptable," while vitamin C is "low." Thiamine is satisfactory, being well within the "high" range, and niacin is also eminently so.

Mashad, one of the principal food-producing areas, showed the best nutrient intakes. The highest caloric, vitamin A, and nutrient intakes were found here. Only Mahabad exceeded it in niacin intake, this being the sole exception to this general statement. The troops in Mashad were the best fed of any of the groups surveyed (Table XXII).

As was expected, more nutrient shortages were encountered at Mahabad and Khash than in any other areas surveyed. Mahabad is in the northwest part of Iran, just south of Lake

TABLE XXIII

Summary Table of Nutrient Consumption at Five Places Surveyed per Man per Day
(Corrected for Conservative Vitamin Losses in Cooking*)

Location	Calories	Protein (gm.)	Fat (gm.)	Calcium (mg.)	Iron (mg.)	Vitamin A I.U.	Thiamine (mg.)	Riboflavin (mg)	Niacin (mg.)	Vitamin C (mg.)
Tehran	3694	135.1	69.0	352.9	26.5	10,451	2.49	0.96	22.2	24.2
Ahwaz	3693	129.6	75.4	500.6	40.1	3,943	2.72	1.21	36.1	18.4
Mashad	4072	145.2	74.4	642.0	40.5	14,420	3.26	1.41	38.3	42.1
Mahabad	4018	143.0	82.1	398.3	36.0	405	3.07	1.24	39.2	10.4
Rasht	3852	133.2	70.4	397.0	33.1	3,300	2.78	1.06	35.7	17.0

* See Table xx.

Rezaiyeh, 175 kilometers from Tabriz. The only transportation route is a rather poor road, so it is very difficult to transport supplies. It thus becomes necessary to depend upon local foods which are usually in short supply. Fresh vegetables were completely lacking in the diet at the time of the survey. Vitamin A intake was lowest of all here, being only about 400 I.U., well down in the "deficient" range. Vitamin C was lowest here also, just on the "deficient" borderline, with a value of 10 mg. Interestingly, the intake of thiamine, niacin, and riboflavin was quite satisfactory. The thiamine and niacin levels were as good as at any of the survey sites. Calcium intake was on the borderline of "low," with an average daily intake of about 400 mg., about the same as at Rasht (Table XXII).

In order to include a "rice-eating area" in the surveys, the next study was carried out at Rasht, southwest of the Caspian Sea. Since recruits are generally allowed to remain fairly close to their homes after induction into military service, the dietary habits of these men reflected the rice-eating habits of the civilian population. The bread issue was smaller at Rasht than elsewhere and the rice issue was correspondingly larger. A second difference in the food offered at Rasht from that of other areas was the serving of fish, readily available from the Caspian Sea. According to the Iranian officers, these men liked fish, in marked contrast to troops from other areas. This was the only area surveyed where fish was served.

Rasht was next to Mahabad in low intakes of vitamin A and C, with both vitamins in the "low" range by ICNND standards, vitamin A being at 3,300 I.U. per day and vitamin C at 17 mg. Rasht was also next to lowest with respect to riboflavin, being only 0.1 mg. more than Tehran, a difference of no practical significance. While the low riboflavin intake at Tehran is apparently due to the difference in flour, the low intake at Rasht seems to be due to smaller intakes of flour and mutton. The calcium intake was similar to that at Mahabad, at the borderline of acceptability (Table XXII).

As indicated earlier, inclement weather prevented the U. S. survey team from including Khash in its itinerary, but the Iranian team made a nutrition survey there after the U. S. team departed. Detailed food intakes were not reported by the Iranian team in the Khash survey, but it was learned that a general effort was made to follow the master menu. However, many items were missing. The troops were spread over a vast area, precluding the use of the large messing establishments found at other army posts. Making a detailed survey turned out to be very difficult. The monthly monetary allowance in the Khash area was 447.20 rials per man (76 rials equals one U. S. dollar), while in other areas it was 550 per month. The weekly meat ration was reported to be 550 gm., compared to 800 gm. in Mashad, 1,065 gm. in Tabriz, 1,240 gm. in Mahabad and 1,370 gm. in Tehran. (It should be pointed out that, according to Table XXI, the meat intake in Tehran under the winter menu, consisting solely of mutton, is 1,275 gm.) The cost of living at Khash is higher than in other parts of Iran. Thus one would expect to find here the poorest nutritional level encountered in any area. This expectation was borne out by biochemical and clinical findings.

It should be remembered that during the time these surveys were made, February through March, fresh foods were in seasonally short supply. The menu as served reflected, of course, what was available locally, and it is doubtless true that during the growing season and even into the fall when some vegetables could be stored, the intake of fresh vegetables and melons and similar foods would be higher. An inspection of a summer master menu confirmed this idea.

The equivalent of the post exchange was found occasionally on some posts. However, any effect on the soldiers' dietary intake may ordinarily be disregarded. An occasional glass of tea is about all that would be available, and the average soldier is not in a financial position to invest even in this luxury very often.

It should be noted that about six months before the survey was undertaken a third meal, breakfast, was added to the ration of the troops of the Iranian Armed Forces. This

TABLE XXIV

Suggested Guide for Interpretation of Nutrient Intake Data (Young Adult Males*)

	Deficient	Low	Acceptable	High
Niacin (mg./day)	5	5–9	10–15	>15
Riboflavin (mg./day)	0.7	0.7–1.1	1.2–1.5	>1.5
Thiamine (mg./1,000 calories)	0.2	0.20–0.29	0.3–0.5	>0.5
Vitamin C (mg./day)	10	10–29	30–50	>50
Vitamin A (I.U./day)	2,000	2,000–3,499	3,500–5,000	>5,000
Calcium (gm./day)	0.3	0.30–0.39	0.4–0.8	>0.8
Iron (mg./day)	6.0	6–8	9–12	>12
Protein (gm./kg.)	0.5	0.5–0.9	1.0–1.5	>1.5
Calories	...	...	...	...

* Prepared by the Interdepartmental Committee on Nutrition for National Defense. These guides are intended to apply to twenty-five year old physically active males of 67 inches (170 cm.) in height and 143 pounds (65 kg.) in weight living in a temperate climate and consuming a varied diet. The quantites specified *should never be considered as inflexible "requirements."* In interpreting nutrition surveys of population groups, average values falling in one or another of the above categories conceal the fact that some individuals will receive more and others less than average. In addition, it is known that there is much variability from one to another individual in their requirement for various nutrients. Variations in body size, activity, climate, types of food available, and other factors modify requirements and, consequently, interpretation of survey data. The nutrient content of food may be altered materially during food preparation, a fact which must always be considered in evaluating dietary intake data.

was done at the suggestion of the Commanding General, U. S. Military Assistance Advisory Group. This additional meal has increased each man's daily food intake. No nutrition survey was made prior to the innovation, thus the degree of improvement in nutriture is not known, and no comparison can be made with the present data.

COMMENTS

The objectives of the nutrition survey were to appraise the nutritional status of the armed forces, define the major problem areas, determine practical means for improvement, train host country personnel in survey technics, and assist in establishing a nutrition research unit for the government of Iran. Nutritional status assessment consisted of three means of appraisal, namely, (1) dietary food intake which recorded food consumed over a limited period (usually not more than seven days), (2) physical examination, and (3) biochemical analysis of blood and urine samples for nutritional deficiency "indicator" signs. Interpretation of the findings must of necessity consider the limitation of any one of these. However, when taken together they provide a basis for evaluation and improvement.

TABLE XXV

Relations Among Biochemical, Clinical and Dietary Findings in the Iranian Armed Forces

Determinations	Results
Clinical examinations (no.)	1,730
Biochemical determinations (no.)	396
Riboflavin nutriture	
With angular lesions (%)	15
With angular scars (%)	21
With urinary excretion <30 μg./6 hr. (%)	21
Average dietary intake (mg./day)	1.1
Thiamine nutriture	
With loss of ankle jerk (%)	1.3
With urinary excretion <25 μg./6 hr. (%)	9
Average dietary intake (mg./day)	2.8
Vitamin C nutriture	
With "scorbutic-type" gums (%)	1.6
With bleeding gums (%)	13
With serum vitamin C <0.2 mg./100 ml. (%)	51
Average dietary intake (mg./day)	22
Vitamin A nutriture	
With follicular hyperkeratosis (%)	30
With serum vitamin A <20 μg./100 ml. (%)	24
With serum carotene <40 μg./100 ml. (%)	58
Average dietary intake (I.U./day)	6,504*

* Range for areas: 405 to 14,420.

TABLE XXVI

Prevalence of Clinical Signs of Borderline Nutrition Among Troops in Khash Versus Other Areas (Per Cent of Troops)

	Khash	Area Having the Lowest Incidence	Area Having the Highest Incidence	Average of the Other Five Areas*
Below 90 per cent "standard weight"	45.5	7.1	17.4	13.0
Low riboflavin intake signs				
Angular lesions	11.1	7.5	19.0	14.6
Angular scars	29.3	17.0	29.8	20.8
Cheilosis	16.5	1.3	6.5	3.4
Nasolabial seborrhea	9.8	0.3	4.5	2.5
Urinary riboflavin excretion, (30 μg./6 hr.)	23.2	4.5	30.7	21.3
		1.3	**0.9**	**1.1**
Low vitamin C intake signs				
Gums, bleeding	16.8	5.5	20.6	12.9
Marginal swelling	23.6	5.1	38.5	21.9
"Scorbutic-type"	3.0	0	3.4	1.6
Marginal redness	21.2	6.5	24.7	13.7
Plasma vitamin C (0.1 mg./100 ml.)	94.0	19.2	44.4	31.6
		30	**17**	**22**
Low vitamin A intake signs				
Follicular hyperkeratosis	46.1	21.4	49.5	29.8
Xerosis	9.8	0	0.3	0.1
Plasma vitamin A (20 μg./100 ml.)	68.1	10.2	50.0	23.7
Plasma carotene (40 mcg./100 ml.)	95.8	18.6	93.8	58.1
		10,451†	**405**†	**6,504**†

NOTE: Bold face figures represent dietary intake (mg./day).
* Tehran, Ahwaz, Mashad, Mahabad, Rasht.
† I.U./day.

As already stated, about six months prior to this survey a major change in the dietary habits of the troops had been made. A third meal, breakfast, was added to the usual two meals of the day. This added ration allowance increased the quantity of food intake per man per day and undoubtedly improved the physical condition of the troops. The degree of improvement cannot be ascertained due to lack of an initial baseline nutrition survey to which the present survey could be compared.

In interpreting the significance of the physical findings, several factors must be kept in mind in regard to relatively mild deficiency states. Even a well nourished population group will show a certain prevalence of most of the physical signs listed, and suboptimal levels of blood and urine biochemistry. Even though the "average" intake of a nutrient may be adequate, there will be persons with higher requirements, or persons who consume less than average amounts and thus be deficient or borderline with respect to the nutrient. Furthermore, few, if any, of these physical signs are "specific" or "diagnostic" of a particular nutrient deficiency since they can be produced by non-nutritional factors or by any one of several nutrient deficiencies.

In general there is good over-all correlation among the three phases of nutritional appraisal, as illustrated by the criteria for riboflavin, thiamine, vitamin C and vitamin A nutriture (Table XXV). This correlation was maintained when one compares the findings of areas having the lowest and highest incidence with Khash (in general a problem area) to the average (Table XXVI). In general the nutritional status as regards calories, protein, iron, thiamine, and niacin was adequate.

Calcium intake was low when compared to the usually recognized standards, although no evidence of calcium deficiency was seen. Studies in other countries have shown adult

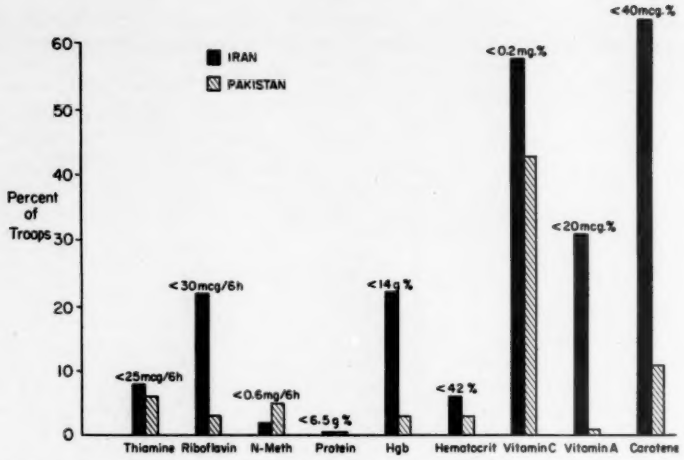

FIG. 4. Per cent of troops in Iran and Pakistan Armed Forces with biochemical nutrient levels below "acceptable" values.

populations with lower intakes of calcium may have no physical signs of deficiency.[37] Such low intakes may be significant in the civilian population, and especially among children and pregnant women, but civilians were not studied in this survey.

Intakes of vitamin A in general were below what are usually considered to be acceptable levels, except for Mashad and Tehran. Similarly, variation between areas in vitamin C intake was noted. Even though the dietary study indicates an excellent intake of vitamin A among the troops in the Mashad and Tehran areas, clinical findings suggest that the troops have over a longer period of time subsisted on lower levels. Clinical signs of vitamin A insufficiency, such as follicular hyperkeratosis, and low plasma vitamin A levels and carotene levels may be in the process of disappearing due to the recent addition of a third meal. Another possibility is that an adequate dietary level of vitamin A intake occurs only sporadically. It is reasonable to suppose that if high levels of vitamin A intake, such as those found in the Mashad area could be maintained, most of the clinical signs usually associated with low vitamin A intakes would disappear.

A comparison of the biochemical results of the surveys of troops in Pakistan and Iran is given in Figure 4. Only those troops with blood and urine levels indicative of deficient nutriture are included. Vitamin C intake in both countries, as judged by biochemical findings, appears to be insufficient. For most other values except protein and N′-methylnicotinamide the status of the troops in Pakistan appears to be better than that of those in Iran.

Rations in the different areas varied considerably in regard to the foods containing vitamins A and C. For example, Tehran rations included spinach, parsley, leeks, coriander, carrots, beets, plums, oranges, and lentils, and of these only carrots and coriander were in the Mahabad ration. If all areas could have a similar amount of these foods, availability of vitamins A and C would be increased significantly.

Dietary levels of vitamin C as low as those in Mahabad have previously been shown to be adequate to prevent scurvy in most adults,[38,39] although higher levels are necessary for maintenance of optimum health. It should be pointed out that vitamin C intake may be even lower than that calculated, due to cooking losses.

Lesions usually associated with mild riboflavin deficiency were widespread; riboflavin intake was generally low. The percentage of extraction of the flour was not known, but was calculated on the basis of little or no extraction for all areas except Tehran, where refined white

flour is used, resulting in a 30 to 50 per cent loss of riboflavin. The relatively high incidence of cheilosis and angular mouth lesions in Tehran correlates well with this high rate of flour extraction.

Progress in Nutrition Since Survey in 1956

The nutrition survey of the Iranian Imperial Army conducted by the ICNND early in 1956 has been followed by significant and progressive developments in Iran. An Armed Forces Iranian Nutrition Committee was established which conducted surveys in the troop garrisons in Khash in 1956 and in Gorgan in 1957. The results of the former survey have been included in the data presented in this report. Prompt action was taken to correct the dietary inadequacies that were found.

The master and area menus have been improved and are analyzed to insure nutritional adequacy. The ration issue has been improved by including a greater variety (and quantity to some areas) of vegetables, fruits, and fish. Hydrogenated vegetable oil has been fortified with vitamin A. Breakfasts were enlarged and improved to provide more energy for physical activities. Special rations were adopted for paratroopers and others who occasionally engage in extraordinarily active duties. Wider use of undermilled wheat flour has been implemented. The ration distribution system has been improved to insure a more equitable distribution of essential foods in widely divergent areas.

Especially noteworthy was the reopening of the "Shahi Cannery" in the fall of 1956. The armed forces, in addition to providing the initial incentive to reopen and expand the cannery, provided the food specifications, quality control, and inspection through their Veterinary Corps. Benefits have been manifold: canned meats, vegetables, and fruits are issued to outlying posts, and used for "field-type" rations, civilian markets are supplied, new food codes and specifications have been established, and equally important, a cash market and employment have been created for local farmers and workers.

Inadequate kitchens have been completely rebuilt in some areas. The army has developed and is using a few mobile kitchens and bakeries. The acquisition of some mobile refrigerators has enabled army units to keep and serve fish as an additional source of protein.

In the Tehran area, highly refined flour has been replaced by a whole wheat flour of about 94 per cent extraction, the size of the loaves has been decreased and the bread is now baked completely through. Cheese has been added to the daily ration to increase further the riboflavin intake.

Since the time of the survey, a modern plant for processing fluid milk and manufactured dairy products equipped by UNICEF has been put into operation on the outskirts of Tehran.

The nutrition laboratory of the Imperial Iranian Armed Forces, in addition to supporting further surveys, has conducted a wide variety of studies of principal Iranian foods, including nutrient composition, nutrient stability, biological evaluation, enrichment, food palatability, and basic reasearch. An experimental poultry production unit was established, producing 20,000 poults per year. This production is being used to supplement hospital rations. A new modern laboratory was placed in operation in late 1959.

Recent nutrition surveys sponsored by the Iranian Nutrition Committee have indicated a marked improvement in the nutritional status of the troops, especially as regards riboflavin, vitamin A, and vitamin C nutriture.

Following the 1956 survey, the government and armed forces of Iran organized a nutrition conference held in November 1956, with representatives from the armed forces of Iraq, Turkey, Pakistan, the United Kingdom, and the United States. As a result of the conference an Armed Forces Internation Nutrition Committee was organized for the purpose of disseminating and discussing mutual problems in food and nutrition. Similar conferences were held in Turkey in 1958 and in Pakistan in 1959.

SUMMARY

A nutrition survey of typical units of the Imperial Iranian Army was conducted in Iran

in early 1956 by a joint Iranian-U. S. team sponsored by the Interdepartmental Committee on Nutrition for National Defense at the request of the Iranian Government. This survey of troops included the determination of average daily nutrient intakes per person, prevalence of clinical signs usually associated with malnutrition (physical and biochemical) as well as the situation with respect to agriculture and food technology in Iran.

A total of 2,027 men received the physical examination, and biochemical studies were made on 488 of these. These figures include the troops examined by the Iranian survey team in Khash. Studies of the troops showed that, in general, their nutritional status was good, that is, it was adequate with respect to calories, protein, iron, thiamine, and niacin.

There was considerable area or location variation as regards riboflavin, and vitamin A and C nutriture. This was substantiated for the most part by the physical examination, blood and urine biochemistry, and dietary intake findings.

Considerable and impressive progress has been made in improving the feeding and nutrition of the troops since the original survey was conducted in 1956.

ACKNOWLEDGMENT

The staff of the present study consisted of the following persons: Field Director, John B. Youmans, M.D., Survey Director, Joseph S. Butts, PH.D.; Deputy Field Director, Cyrus E. French, PH.D.; Conducting Officer, Col. Morteza Tadayon, M.D.; Clinicians, John H. Browe, M.D.; Lt. Kazem Eghtesady, M.D.; Lt. Taher Semnan Azami, M.D.; Lt. Mesbah Balaghy, M.D.; Lt. Hushang Beizaie, M.D.; Lt. Amanolah Missaghi, M.D.; Lt. Mohamad Movah Hedi, M.D.; Lt. Hashem Sadoughian, M.D.; Lt. Yahya Negahdarie Pour, M.D.; Lt. Col. Laurence M. Hursh, M.D.; Nutritionists, Col. Rahmapollah Deyhimi, D.V.M.; Maj. Ali Khan Afrakhteh; Biochemists, Philip L. White, D.SC.; C. Frank Consolazio, Brig. Gen. Solyman Deyhimi, D.V.M.; Food Technologist, David B. Hand, PH.D.; Agricultural Economist, Arthur G. Peterson, PH.D.; Graduate veterinarians assisting in the laboratory, Col. Amir Khatai, Capt. Djalal Pezeshk, Capt. Djahan Pir Moradi, Capt. Abdal Moshiri, Lt. Vaezadh; Chemists from the Food and Drug Laboratory, University of Tehran, Assad Ghazizadeh, Safad Hatami, Miss Nasnit Sarkiscian; Laboratory technicians from U. S. Army Medical Research and Nutrition Laboratory, R. K. Bailey, W. A. Yergeau.

Col. (now Brig. Gen.) Morteza Tadayon of the Surgeon General's Office, Imperial Iranian Army, was in charge of the arrangements for carrying out the several surveys under the over-all direction of Major General Abdol Karim Ayadi, Personal Physician to His Imperial Majesty the Shah. Brig. Gen. Solymon Deyhimi, Commanding Officer of the laboratory, was responsible for the arrangements for the laboratory, which was completely reconditioned prior to the arrival of the survey team.

The Nutrition Survey Team wishes especially to thank Maj. Gen. Ayadi, M.D., Physician to His Imperial Majesty, and the then Surgeon General, Maj. Gen. Seifollah Parsa, whose wholehearted interest and cooperation contributed greatly to the success of the survey. The assistance of the following officers is also highly valued: Maj. Gen. Ahmad Vosough, Minister of War; Brig. Gen. Rahmatollah Fathi, Chief, Animal Affairs Department; Brig. Gen. Asadoliah Sanii, Quartermaster General; Brig. Gen. Majin, Deputy G-4; and Col. Masih Daneshvar, Office of the Surgeon General. The brigade and division commanders, their subordinates and the medical officers in each location where surveys were made also deserve commendation for their enthusiastic contributions to the success of the survey.

U. S. military personnel who provided invaluable guidance and assistance included the late Maj. Gen. Robert A. McClure, Commanding General, MAAG; Col. E. A. Machen, Jr., Deputy Chief of Mission and Chief of Staff; Lt. Col. U. L. Throm, Medical Advisor to the Imperial Iranian Army; and Lt. Col. V. L. Lewis, Quartermaster Advisor to the Imperial Iranian Army. Thanks are also due to Lt. Col. (now Col.) Laurence M. Hursh and Dr. Theodore Friedemann, formerly of the U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado, for their cooperation in analyzing Iranian food items.

Iranian and American civilian officials who contributed materially to the success of the survey included Dr. Jahanshah Saleh, Minister of Health; Dr. Shams-El-Din Mofidi, Assistant Director, Institute of Malariology, University of Tehran; Mr. Amir Parviz, Head, Department of Agricultural Economics, Ministry of Agriculture; Mr. Clifford C. Matlock, Special Assistant to the American Ambassador; and Mr. William R. Hatch, the American Agricultural Attaché. Personnel of the International Cooperation Administration (Point IV) staff who provided considerable assistance included Dr. Albert P. Knight, Chief, Health Division; Mr. Ray Johnson, Chief, Agricultural Division; Mr. Harris H. Richard, Advisor on Food Processing; Mr. Dean W. Hubbard, Agricultural Economist; and Dr. Thomas S. Moulding, Health Division.

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ASCN News

Annual Meeting
American Society for Clinical Nutrition

THE first annual meeting of the American Society for Clinical Nutrition convened at 1 o'clock in the Music Room of the Chalfonte Hotel at Atlantic City on April 29, 1961, Dr. Richard Vilter, president, presiding. The minutes of the November 1960 Council meeting were read and approved.

The Secretary called the attention of the Society to an inadvertent omission from the Constitution. According to the minutes of the May 1, 1960, meeting, page 7, it had been agreed that the officers of the Society would consist of the President, Vice President-President-elect, immediate past President and three Council members. Reference to the immediate past President was omitted in drafting the Constitution. The Secretary requested permission of the Society to add this phrase to the Constitution without the formality of a constitutional amendment. This was agreed upon unanimously. The Treasurer's report was read and approved.

Dr. S. O. Waife presented the Editor's report and related the history of *The American Journal of Clinical Nutrition*. Dr. Robert Olson next discussed editorial policy and called attention to the fact that the number of pages of the Journal will be increased during the next six months in order to accommodate an increased load of manuscripts. Beginning January 1, 1962, the Journal will commence monthly publication.

The Auditor's report was heard and approved. Dr. Charles Davidson presented the slate of the Nominating Committee. For President they proposed Robert E. Olson; for Vice President-President-elect, William B. Bean; Secretary-Treasurer, Robert E. Hodges; for Council, Willard A. Krehl, Robert S. Goodhart and George V. Mann. It was moved that nominations cease and that the candidates be elected unanimously. This was seconded and approved.

Dr. Robert Kark, chairman of the Membership Committee, presented twenty-seven new candidates who had been cleared by the Membership Committee of the American Society for Clinical Nutrition. Their names were placed in nomination, and they were unanimously elected to membership. Dr. Laurance Kinsell discussed the question of accepting members who hold a PH.D. degree but do not hold an M.D. degree. After discussion it was agreed that the Constitution provides for such members and that this represents the wish of the Society.

Dr. Richard Vilter gave the presidental address in which he discussed the sequence of events leading to the formation of the American Society for Clinical Nutrition. Full credit was given to Dr. Robert S. Goodhart as an organizer of this movement.

Announcements included plans for the next annual meeting to be held in Atlantic City on April 28, 1962. The dues and assessments were unchanged. However, the cost of the Journal will increase because of monthly publication beginning January 1, 1962. The question of creating a committee for study of nutrition education was deferred to the Council. Following the business meeting, the scientific session proceeded from 1:30 to 5:00 P.M.

R. E. HODGES, M.D.
Secretary

New Members

Elected April 29, 1961

ARTHUR F. ABT, M.D.
Veterans Administration Center
Martinsburg, West Virginia

MOISÉS BÉHAR, M.D.
Director, Institute of Nutrition for Central America and Panama
Apartado Postal No. 11-BB
Carretera Roosevelt, Zona 11
Guatemala, C.A.

BACON F. CHOW, PH.D.
Department of Biochemistry
School of Hygiene
Johns Hopkins University
Baltimore 5, Maryland

WILLIAM W. FALOON, M.D.
State University of New York
University Hospital of the Good Shepherd
Department of Medicine
150 Marshall Street
Syracuse 10, New York

SAMUEL J. FOMON, M.D.
Department of Pediatrics
University Hospitals
State University of Iowa
Iowa City, Iowa

IRA GORE, M.D.
Chief, Laboratory Service
Veterans Administration Hospital
Veterans of Foreign Wars Parkway
West Roxbury 32, Massachusetts

ARILD E. HANSEN, M.D.
Director of Research
Bruce Lyon Memorial Research Laboratory
Children's Hospital of the East Bay
Grove and 51st Streets
Oakland 9, California

D. MARK HEGSTED, PH.D.
Department of Nutrition
Harvard University
School of Public Health
1 Shattuck Street
Boston 15, Massachusetts

MAX K. HORWITT, PH.D.
L. B. Mendel Research Laboratory
Elgin State Hospital
750 South State Street
Elgin, Illinois

JAMES IACONO, PH.D.
Department of Medicine
University of Cincinnati
Cincinnati, Ohio

JOSEPH A. JOHNSTON, M.D.
Henry Ford Hospital
Detroit, Michigan

ANCEL KEYS, PH.D.
Laboratory of Physiological Hygiene
University of Minnesota
Stadium Gate 27
Minneapolis 14, Minnesota

WILLIAM H. MARONEY, M.D.
Commanding Officer
U.S. Army Tropical Research Med. Lab.
San Juan, Puerto Rico

MARGARET A. OHLSON, PH.D.
Department of Nutrition
University Hospitals
Iowa City, Iowa

HANS POPPER, M.D.
The Mount Sinai Hospital
5th Avenue and 100th Street
New York 29, New York

MILTON E. RUBINI, M.D.
Chief, Department of Metabolism
U.S. Army Tropical Research Med. Lab.
San Juan, Puerto Rico

NEVIN S. SCRIMSHAW, M.D., PH.D.
Director, Pan American Health Organization
INCAP, Guatemala City, Guatemala

JOSEPH SEITCHIK, M.D.
Hahnemann Medical College and Hospital
230 North Broad Street
Philadelphia 2, Pennsylvania

STANLEY A. TAUBER, M.D.
6357 Farnsworth Street
Philadelphia 24, Pennsylvania

JOHN B. YOUMANS, M.D.
Army Medical Research & Development Command
Main Navy Building
Washington 25, D.C.

Reviews of Recent Books

The Thyroid Gland. *British Medical Bulletin*, vol. 16, no. 2, May 1960. The British Council, London, $3.25.

This bulletin on the thyroid gland is filled with meaty data and rendered especially significant since the fifteen papers it contains are written by British authorities who are conducting original research on various aspects of the thyroid gland. The authors judiciously analyze recent physiologic and biochemical studies on the thyroid gland; these have been made possible by the application of chromatography and radioactive isotopes.

The technics of immunology are adding to the progress of diseases of the thyroid gland. Doctors Roitt and Doniach describe the presence of circulating thyroid antibodies in various diseases of the thyroid. Thus, it would appear that the pathogenesis of some types of chronic thyroiditis in man is related to autoimmunization against thyroglobulin of the patient's own thyroid gland. This opens up a new field on the pathogenesis of a number of human diseases that are attributed to the effect of autoantibodies.

The discussion of "Biosynthesis of the Thyroid Hormone" by Pitt-Rivers, who played such a large part with Gross in the discovery of triiodothyronine, makes for stimulating reading.

The merits of radioactive iodine in the diagnosis of thyroid disorders are critically reviewed by Goolden who possesses not only wide knowledge of the basic knowledge of thyroid metabolism, but also clinical acumen which makes this section valuable to the practicing physician.

The thyroid nodule and particularly its relation to thyroid malignancy has been the subject of prolonged and vehement controversy. It is refreshing, therefore, to read the viewpoint of Taylor who states in his section on the "Genesis of the Thyroid Nodule" as fol lows:

"It should be clear by now that many problems still remain to be answered as to why the diffuse hyperplasia of the thyroid at puberty should become nodular with the passage of time in those individuals who are denied an adequate intake of iodine in their youth. There is no problem, however, in preventing the formation of nodules in the thyroid gland for, if the daily intake of iodine is maintained at 100/200 μg./day, such changes do not occur. There are no recorded adverse effects in man from adding 50–100 μg. of iodine to the daily diet, preferably by iodizing salt, but although the Medical Research Council recommended this measure in Great Britain in 1944 and again in 1948 (Medical Research Council, 1944, 1948), it has never been implemented.

Nodular goitre is a preventable disease. Surely it ought to be prevented."

Because of our major interest in the association of hypercholesterolemia with coronary heart disease, the section by Boyd and Oliver on "Thyroid Hormones and Plasma Lipids" is of special significance. That thyroid therapy will reduce blood cholesterol levels is well known However, there is clinical evidence to indicate that therapeutic doses of thyroid may be harmful: elevate total body metabolism and further aggravate or provoke angina pectoris. Our major concern centers on the problem of lowering blood cholesterol without elevation of body metabolism. Some of the thyroid effects can be dissociated by altering the chemical structure of the basic thyroxin molecule resulting in a thyroid analogue. A good many thyroid analogues are being studied today. Some of these analogues, when given in proper doses, will lower blood cholesterol without affecting total body metabolism. Several papers appeared dealing with the clinical potentialities of such analogues, particularly dextroisomers. The reviewer well remembers the excellent talk on a similar topic given by the junior author in Philadelphia in February 1960.

The remaining articles in the Bulletin are equally good and meet high scientific standards. This Bulletin will prove to beuseful not only to the thyroidologists but also to the practitioner who is interested in the newer thinking in thyroid physiology and its practical application. M. G. WOHL

Yearbook of Endocrinology, 1959–1960, edited by G. S. Gordan. The Year Book Publishers, Inc., Chicago, 1960, pp. 384, $8.00.

Physicians should be grateful to Dr. Gordan for a superb task of selecting and integrating outstanding contributions in the broad field of endocrinology. The summaries of articles selected for inclusion in the Year Book are prepared with excellent completeness, and these articles themselves are drawn from the medical literature of many countries. The customary format of the book arranges the summaries into groups beginning with suprasellar influences followed by adenohypophysis, thyroid, parathyroid and calcium metabolism, carbohydrate metabolism, adrenal, reproductive system and neoplasia. The pertinent and stimulating comments by the editor interspersed throughout each section provide added interest to the perusal of this useful volume.

Nutritionists will find a ready source of information relating the newer work in endocrinology and selected topics in metabolism to nutrition. C. R. SHUMAN

Clinical Studies in Nutrition, by Eleanora Sense. J. B. Lippincott Co., Philadelphia, 1960, pp. 249, $4.00.

This textbook, written for the student nurse, is divided into two parts. The first is an introduction to clinical nutrition and deals with such topics as nutrition ward walks and conferences, communication in nutrition, nutrition rehabilitation, protective agencies for nutrition. The second part relates nutrition to specific diseases such as those of the gastrointestinal system, circulatory system and skin. Patient studies are presented as illustrations.

The author attempts to cover too much material which results in oversimplification and a tendency to superficiality. Little material is devoted to the physiologic principles of nutrition. The emphasis is instead on psychologic aspects involved in the nutritional education of the patient. This paperback book contains no illustrations, figures or charts, although an appendix is included which lists sample diets, a method of dietary analysis, and the recommended daily dietary allowances. Each chapter is concluded with a short bibliography.

M. W. Bates

A Primer of Water, Electrolyte and Acid-base Syndrome, edited by E. Goldberger. Lea & Febiger, Philadelphia, 1959, pp. 332, $6.00.

This practical and informative text deals with the management of many medical and surgical problems. Despite a plethora of books dealing with this subject, disturbances in fluid balance continue to provide a serious stumbling-block in the care of patients. A useful discussion of the basic physiology involving water, electrolytes and osmolarity of body fluids and their interrelationships prepares the reader for the clinical presentations which follow. The syndromes dealing with water and electrolyte imbalance are defined individually, and described as to pathologic physiology, symptoms, signs, diagnosis and treatment. Emphasis is laid on interpretation of acid-base balance in proper biochemical terms. The importance of the carbon dioxide capacity and carbon dioxide content in the differentiation of respiratory and metabolic acidosis or alkalosis is described with references to appropriate nomograms. The clinical syndromes of renal tubular acidosis, diabetic acidosis, uremia, disturbances in potassium metabolism and a wide variety of conditions encountered in medical practice are elucidated. The latter sections provide information on the management of surgical patients, infants and children; solutions used for fluid therapy; and calculations of fluid and electrolyte requirements.

This text can be recommended for use by physicians and students for obtaining the most recent information concerning the diagnosis and treatment of fluid and electrolyte disturbances.

C. R. Shuman

Books Received for Review

Books received for review by *The American Journal of Clinical Nutrition* are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for extensive review.

Diagnostic Stomatology. A Clinical Pathologic Approach, by E. Cheraskin. McGraw-Hill Book Co., Inc., New York, 1961, pp. 338, $12.50.

Food, Nutrition and Diet Therapy, 3rd ed., by Marie V. Krause. W. B. Saunders Co., Philadelphia, 1961, pp. 716, $6.75.

Performance Capacity—A Symposium, edited by H. Spector, J. Brozek and M. S. Peterson. National Academy of Sciences—National Research Council, Washington, D. C., 1961, pp. 257.

Metabolic Effects of Adrenal Hormones. Ciba Foundation Study Group No. 6, edited by G. E. W. Wolstenholme and M. O'Connor. Little, Brown & Co., Boston, 1961, pp. 109.

Adrenergic Mechanisms, Ciba Foundation Symposium, edited by G. E. W. Wolstenholme and M. O'Connor. Little, Brown & Co., Boston, 1961, pp. 632, $12.50.

Annual Review of Medicine, Vol. 12, edited by David A. Rytand. Annual Reviews, Inc., Palo Alto, 1961, pp. 453, $7.00 (U.S.A.), $7.50 (elsewhere).

Abstracts of Current Literature

CURRENT PROGRESS IN ARTERIOSCLEROSIS

The cholesterol-lipid-lipoprotein concept of pathogenesis of arteriosclerosis is based on the hypothesis that elevated dietary fat intake represents the major factor in atherogenesis. However, there are many weak links in the chain of evidence cited to support this concept. Among the epidemiologic studies which dispute the nutritional theory are the following:

A Population Study—Atherosclerosis. D. Groom. *J. Am. Dietet. A.*, 35: 919, 1959.

This study compares the autopsy findings in 139 Negro adults at the Medical Center Hospitals of the Medical College of South Carolina with the autopsy findings in 128 patients from the Hospital General of Port-au-Prince, Haiti. The main purpose of the investigation was "to measure the prevalence of atherosclerosis in the coronary arteries and aortas of representative samples of these two populations and to do this by pathologic rather than clinical criteria."

The severity of atherosclerosis at autopsy was graded according to the number of plaques in the arteries and the resultant degree of occlusion of the lumen, and the severity of the disease in the aorta was gauged by the number and size of plaques.

Since the two populations studied were of common racial origin the author believes that "any observed inequality in incidence of coronary disease might. . . be properly attributed to differences in environment—whether that be primarily diet, stress, climate or whatever."

Pathologic evaluation of the degrees of coronary and aortic atherosclerosis revealed that the American Negros had almost double the average severity of coronary disease of the Haitians. This held true for both men and women and at all age decades over twenty. No comparable differences were observed in the aortas of these subjects. The author believes this lack of differences indicates the importance of factors other than diet in the genesis of atherosclerosis.

The age distribution of the two populations is interesting. Among the Haitians, 67 per cent were twenty to fifty years of age whereas 34 per cent of the South Carolinians were in this age group. The Haitians numbered 26 per cent and the South Carolinians 53 per cent in the fifty to seventy year age groups. The remainder or 16 and 13 per cent, respectively, were in the seventy to ninety-nine year age group. This difference in age distribution may have had some effect on the average score mentioned although the author made comparisons decade by decade. J. M. SMITH

The Biochemical Syndrome of Nutritional Arteriosclerosis. J. Enselme. *Ann. Nutrition*, vol. 13, no. 4. 1959.

The author describes the biochemical syndrome of nutritional atherosclerosis. He successively studies facts relating to anomalies of the lipids: cholesterol, phospholipids, glycerides and lipoproteins. He discusses the first studies on glucoproteins and points out that a more extensive study of serum holoproteins and of cellular permeability will probably yield interesting results in the future. H. GOUNELLE

Cardiac Infarction in the Bantu. V. Schrire and C. J. Uys. *Am. J. Cardiol.*, 2: 453, 1958.

Three cases of myocardial infarction in the Bantu, proved at autopsy in Cape Town, South Africa, are reported in detail. Only one of these was associated with extensive atheroma. One was associated with severe hypertension, the other with syphilitic aortitis and incompetence of the aortic valve.

These three cases represent the total autopsy incidence of myocardial infarction in the Bantu during a seven-year period comprising 162 proved myocardial infarcts in Europeans and Cape Coloureds. The Bantu racial group accounted for 11.4 per cent of the total autopsies performed. W. H. ABELMANN

Effects of Periodic Mental Stress on Serum Cholesterol Levels. S. M. Grundy and A. C. Griffin. *Circulation*, 19: 496, 1959.

Two groups of medical students, comprised of fifty and forty-seven men, respectively, were studied. Total serum cholesterol values were determined in serum obtained in the postabsorptive state during the middle of the teaching sessions and again on the first day of final examination.

The control values averaged 213 and 215.7 mg. per 100 ml. for the two groups, and rose significantly to 248.2 and 239.4 mg. per 100 ml. during the examination period. In 50 and 44 per cent of the students the increase was greater than 25 mg. per 100 ml.

W. H. ABELMANN

Atherosclerosis, Disease or Syndrome? An Attempt at Classification. J. de Brux. *Presse méd.*, 67: 813, 1959.

It is difficult to consider atherosclerosis as a morbid entity with a definite onset, full blown clinical state and development. There is not *one* atherosclerosis but a number of such conditions, the only slightly different histologic forms of which probably do not correspond to identical etiological processes, at least in their terminal stage. Atherosclerosis presumably stems from a physiologic disequilibrium between deposit of glycolipoprotein in the plasma and the processes of lysis; there are many causative factors that can disrupt the normal equilibrium of these substances. The danger is not apparent until the process has become irreversible or too intense. H. GOUNELLE

Cytochemical enzyme studies on blood vessels have been initiated revealing variations in localization of esterases (ATPase) and glycolytic enzymes which may be important in the accumulation of lipids within certain areas in the vessel wall.

Relationship of Lipolytic and Esterolytic Activity of the Aorta to Susceptibility to Experimental Atherosclerosis. T. Zemplenyi, Z. Lojda and D. Grafnetter. *Circulation Res.*, 7: 286, 1959.

The lipolytic activity of the aorta was compared in male rats, rabbits, hamsters, guinea pigs and cocks. Lipolytic activity was measured in terms of the amount of nonesterified fatty acids freed during incubation of finely divided fresh aorta with lipemic human serum. The data reported indicate that the lipolytic activity of the aorta is significantly greater in the rat than in the rabbit, cock or guinea pig.

Histochemical estimation of nonspecific (simple) esterase and A-S esterase (esterase splitting naphthol-AS acetate) by an azo-coupling method was carried out in the aortas of rats, rabbits, hamsters and guinea pigs. The localization of esterases was always the same in a given species, but differed between species. In the rat aorta, there was intense esterase activity in the media and in connective tissue cells of the adventitia, while the intima reacted only occasionally. The rabbit aorta reacted similarly but much less intensively. In the hamster, adventitial fibrocytes reacted strongly, while media and intima showed only weak reactions. In the guinea pig, there was marked activity in endothelial cells and adventitial fibrocytes, and only weak staining of the media.

The implications of the data for species susceptibility to atherosclerosis are discussed.

W. H. ABELMANN

Studies of Fat Lipolysis by Post-Heparin Human Plasma Lipoprotein Lipase and by Human Pancreatic Lipase. H. Engelberg. *Circulation*, 19: 884, 1959.

The rate of lipolysis of various fat substrates in human plasma obtained from fasting subjects after the administration of heparin was studied by determining the release of unesterified fatty acids. Such plasma samples from twelve of fourteen subjects showed a higher rate of lipolysis of unsaturated triglyceride lipoproteins than of saturated (cream) lipoproteins. In each of five experiments, using aliquots of citrate eluates of tricalcium phosphate adsorbates of plasma from nonfasting subjects after the administration of heparin, lipolysis of lipoprotein of vegetable origin was more rapid than that of cream. Similar results were obtained when human pancreatic lipase was used as the fat-splitting enzyme.

The author proposes that more efficient activity of heparin lipoprotein lipase upon unsaturated fat substrates may account for the hypercholesterolemic and hyperlipoproteinemic effect of animal (saturated) fats in man. W. H. ABELMANN

Saturated and Unsaturated Fats. Effects on Cholesterolemia and Atherogenesis in Chicks on High Cholesterol Diets. J. Stamler, R. Pick and L. N. Katz. *Circulation Res.*, 7: 398, 1959.

The effects of supplementary saturated and unsaturated oils were studied in young growing cockerels on high cholesterol (0.5 to 2 per cent) diets for periods of five to fifteen weeks. Plasma total cholesterol values and evaluation of gross lesions of the aorta and of microscopic coronary lesions are reported.

Hypercholesterolemia and the patterns of atherosclerosis were similar in the groups fed various unsaturated oils and in the groups fed various saturated fats (5 or 10 per cent). Unsaturated oils failed to suppress hypercholesterolemia and atherogenesis. Cholesterol-fed cockerels given supplements of fats and oils high in oleic acid tended to show slightly lower serum choles-

terol levels, while cockerels given oleic acid *per se* showed less atherosclerosis. W. H. ABELMANN

The mucopolysaccharides of the blood vessel have a binding capacity for calcium and other electrolytes. This binding activity may account for the retention of lipids within an area of increased mucopolysaccharide deposition.

Radiographic Study of Aortic Plaque Formation. I. G. Fels. *Circulation Res.*, 7: 693, 1959.

The ability of the human aorta to bind calcium ion was studied. Pieces of thoracic aorta were extracted with acetone-petroleum ether and decalcified. The tissue was then incubated in a solution of Ca^{45} Cl_2, washed and radioautographed.

In sclerotic aortic tissue, the uptake of Ca^{45} was specifically confined to the positions of plaques. Binding of calcium was localized primarily in the intima. Binding was demonstrated in the aorta of a twenty-three months old male infant. Much of the binding ability was lost upon boiling.

The influence of bound calcium upon the stability of emulsions was also studied. After recording the Ca^{45} binding pattern, aorta was incubated with an emulsion containing oleic acid-I^{131}, and another radioautograph was made with a short exposure. The radioactive oleic acid was found deposited in the sites which bound Ca^{45}. Emulsions containing cholesterol-C^{14} were also studied.

The author concludes that the binding of calcium has a marked effect upon the stability of emulsions, causing precipitation. W. H. ABELMANN

Passage of Labeled Cholesterol into the Aortic Wall of the Normal Dog. L. E. Duncan, Jr. and K. Buck. *Circulation Res.*, 7: 765, 1959.

Dogs were force-fed gelatin capsules containing cholesterol-4-C^{14} in sesame oil, and sacrificed at varying times. Serum labeled cholesterol was determined, and the inner, middle and outer layers of different portions of the thoracic and abdominal aorta were analyzed for radioactivity.

The serum concentration of labeled cholesterol rose rapidly to a peak between one and three days after injection and then decreased gradually. From three days on radioactivity could be detected in the aortic wall. Initially the ratio of tissue concentration to serum concentration for the inner layer of the aorta rose most rapidly in the ascending aorta; the rise was progressively less down the length of the aorta. Between ten and forty days the rates of increase of the ratios changed so that the ratios became greater distally than proximally. The ratios became constant at about forty days, forming a gradient with the highest value at the distal end and the lowest value at the proximal end of the aorta. The ratios for the middle and outer layers rose with time and became constant at about forty days; there was no systematic difference between sites.

Labeled cholesterol entered the abdominal aorta only one sixth as fast as it entered the thoracic aorta. At each level of the aorta, labeled cholesterol entered at about one third the rate of albumen.

The authors suggest that cholesterol is carried into the aortic wall of the normal dog as part of normally occurring lipoprotein molecules. W. H. ABELMANN

Experimental Studies Concerning the Role of Vegetable and Animal Dietary Fat in Arteriosclerosis. H. Holzner, E. Kriehuber, and R. Wenger, *Arch. path. Anat.*, 333: 210, 1960.

Young rabbits weighing about 2,000 gm. received a stock diet supplemented with (1) 1 gm. cholesterol, (2) 1 gm. cholesterol and 10 gm. olive oil, (3) 10 gm. olive oil only, (4) 1 gm. cholesterol and 10 gm. lard, (5) 10 gm. lard only. The fat-enriched diets did not agree with the animals, and they died of diarrhea. Therefore, in another group the supplements of lard and olive oil were reduced to 5 gm. The rabbits thus treated were observed for periods up to three months. At necropsy, the aortas were studied for the occurrence of arteriosclerosis. The findings indicate that in the rabbits fed cholesterol more severe arteriosclerosis developed than in those fed lard whereas those fed olive oil had only minor aortic changes. Addition of lard to the cholesterol-containing diet accentuated and supplements of olive oil decreased the severity of the vascular lesions. Vegetable fat thus had a protective effect on the evolution of arteriosclerosis. M. SILBERBERG

Localization and Retention of Triolein I^{131} in Various Tissues of the Atherosclerotic Rabbit. L. Felton, M. Friedman, S. O. Byers and P. Cady. *Am. J. Physiol.*, 197: 351, 1959.

The rate of disappearance of intravenously injected triolein I^{131} from the blood stream and its concentration in various tissues were studied both in the normal and in the atherosclerotic rabbit. No difference in the rate of disappearance of the I^{131} from blood was observed between the normal and atherosclerotic rabbit. The atherosclerotic aorta was observed to take up approximately twice as much I^{131} as normal aorta when the tissues were assayed ten minutes after injection. However, twenty-four hours after injection no essential difference was observed. All other tissues examined (fat, adrenal, spleen and liver) were observed to take up and retain more I^{131} than either the normal or the atherosclerotic aorta. The results suggest that the atherosclerotic aorta of the rabbit, while taking up initially more I^{131} than the normal aorta, appears to metabolize or otherwise rid itself of such I^{131} as rapidly as the latter in a period of twenty-four hours. AUTHORS

Coronary Atheromatous Changes Induced by Chronic Hypercholesterolemia in Dogs. G. L. Jordan, Jr., M. E. DeBakey and B. Halpert. *Am. J. Path.*, 35: 867, 1959.

This article deals with the findings in aortas and cor-

onary arteries of dogs used previously for the study of arterial grafts. The animals were treated with I^{131}, and fed a diet supplemented with cholesterol for periods from five to fourteen months. The serum cholesterol rose to values ranging from 735 to 1,770 mg. per 100 ml. In thirty-nine of eighty-four male or female dogs atheromas developed in the aortas, and in thirteen of these thirty-nine they also developed in the coronary arteries. Myocardial infarcts were not observed. Why these atheromatous lesions occurred only in a limited number of animals has not been discussed or explained.

Several questions arise regarding the results obtained. (1) The condition cannot be referred to as "ablation" of the thyroid without considerable restrictions. The degree of destruction of the thyroid by I^{131} varies remarkably and can be evaluated only by microscopic studies. However, pertinent data are not given. (2) Regenerating thyroid nodules develop rather frequently in the thyroid region, a condition that should be investigated in every case. However, there is no information about this point. (3) Thyroid function varies from strain to strain and depends upon sex and age. Such factors are known to influence the effectiveness of the treatment with I^{131}. In view of these complicating factors it is hard to see why use of I^{131} should be more suitable for the study of atherosclerosis than surgical removal of the thyroid as has been carried out in dogs by many investigators. M. SILBERBERG

Correlations between lipid abnormalities in serum and incidence of arteriosclerosis are often cited to support the nutritional theory of atherogenesis. However, it is equally possible that lipid changes derive from disease states which affect blood vessels independently and simultaneously.

The Distribution of Lipid and Phospholipid in Paper Electrophoresis of the Serum Lipoproteins in Normal Subjects and in Patients with Atherosclerosis. M. A. Chapin and S. Proger. *J. Lab. & Clin. Med.*, 53: 39, 1959.

Total lipids and phospholipids were measured in serum beta- and alpha-lipoproteins which had been separated by paper electrophoresis. The serum specimens studied were obtained from small groups of young men, young women, older people and about twenty patients with atherosclerosis. A few patients with diabetes mellitus, nephrosis, myxedema and essential hypercholesterolemia were studied. More lipid with relatively smaller amounts of phospholipid in beta-lipoprotein and less lipid with relatively larger amounts of phospholipid in alpha-lipoproteins were found when the serum specimens from young men were compared with those from young women. The serum specimens from the patients with atherosclerosis showed more total lipid and phospholipids in beta-lipoproteins than those from young women. The lipid content of the alpha-lipoprotein fraction in these patients was somewhat lower with relatively more phospholipid than that in young women. Similar patterns were found in the few subjects with diabetes, nephrosis, etc. studied.

G. HOLLIFIELD

Dietary Fat, Serum Cholesterol Levels and Incidence of Atherosclerosis in Delhi. S. Padmavati, S. Gupta and G. V. A. Pantulu. *Circulation*, 19: 849, 1959.

Fat intake, total serum cholesterol levels, and clinical and electrocardiographic evidence of coronary atherosclerosis were evaluated in one hundred men and twenty-four women of high socioeconomic status and in twenty-six urban and twenty-two rural men of low socioeconomic standing.

In the high income group the serum cholesterol levels increased with age and with increasing intake of fat, both of these features being absent in the low income group. In both groups serum cholesterol levels rose with increase in body weight. Clinical data suggest but do not establish a lower incidence of atherosclerosis in the group of low socioeconomic status.

W. H. ABELMANN

Diagnosis of Atherosclerosis. I. Correlation Between Clinical Diagnosis, Serum Cholesterol and Low-Density Lipoproteins, and Resting and Exercise Electrocardiograms. H. Engelberg. *Am. J. Cardiol.*, 1: 315, 1958.

Electrocardiograms at rest and after exercise, serum cholesterol and ultracentrifugal lipoprotein levels of 560 adult private patients were analyzed. Serum cholesterol and lipoprotein levels were generally elevated in 149 patients who had experienced a myocardial infarction, the latter more predictably so. Both levels were also elevated in twenty-seven patients with clinical peripheral arteriosclerotic disease.

The mean serum cholesterol and lipoprotein levels of forty-six patients with hypertension and coronary or cerebral complications were significantly higher than those of fifty-four patients with uncomplicated hypertension. Again the lipoprotein level showed greater separatory power between the two groups than the serum cholesterol.

The lipid measurement correlated directly with the severity of the electrocardiographic findings at rest and during exercise. W. H. ABELMANN

Myocardial Infarction in Rats Fed Diets Containing High Fat, Cholesterol, Thiouracil, and Sodium Cholate. W. A. Thomas and W. S. Hartroft. *Circulation*, 19: 65, 1959.

The authors present an experimental method to produce, by dietary means, cardiac and renal infarcts in male rats. Seven groups of ten rats each were fed diets of varying composition. Myocardial and/or renal infarcts were found in eleven of twenty rats surviving for four to fourteen weeks on diets adequate in protein, minerals and vitamins, containing propylthiouracil, cholesterol and cholic acid and 40 per cent butter or lard.

The infarcts closely resembled cardiac and renal infarcts in man. Abnormal deposition of strainable fat in all layers of walls of coronary arteries was frequent, but mural plaques, intimal or medial fibrosis were not found. Control of individual dietary factors was insufficient to evaluate their role in the production of disease.

W. H. ABELMANN

Lipid-Bound Glutamic Acid Deficiency in Aging Arteriosclerotic Subjects. R. E. Hamilton and L. O. Pilgeram. *Proc. Soc. Exper. Biol. & Med.*, 103: 574, 1960.

Plasma of normal subjects (average age twenty-seven years) was found to contain 33.9 μM/L. of lipid-bound glutamic acid while that of a group of patients with proved but not recent cardiac infarction (average age fifty-six years) contained 17 μM/L. Of the lipid-bound glutamic acid forty-seven per cent was present in the alpha-lipoprotein fraction in the normal subjects, in contrast to 35 per cent in the patients with arteriosclerotic heart disease.

Glutamic acid has been shown to have a thrombokinase inhibitory action, and it is possible that a deficiency of lipid-bound glutamic acid was associated with abnormal coagulability of the blood in the patients who sustained coronary thromboses. Unfortunately the control group was not matched for age or weight.

G. WALKER

Alimentary Lipaemia and Heparin Clearing in Ischaemic Heart Disease. J. R. A. Mitchell and B. Bronte-Stewart. *Lancet*, 1: 167, 1959.

Intravenously administered heparin is known to have a clearing effect on alimentary lipemia, less effective in persons with athroma than in those without, but the possible modes of action have not been fully investigated.

Twelve subjects with ischemic heart disease and twelve age-matched control subjects were given a meal containing 75 gm. of fat. Examination of their plasma showed a greater rise of postprandial lipemia in those with heart disease. Two days later, after ingestion of a nonfatty meal, the same twenty-four subjects were each given 15 mg. of heparin intravenously. Plasma samples obtained fifteen minutes later were tested for their capacity to clear the lipemic samples obtained two days before after the fatty meal. Both types of lipemic plasma were each mixed with heparinized plasma from both groups of subjects; there was no difference.

It is suggested that the difference in postprandial lipemia is due to differences of fat absorption rather than to differences of removal from plasma. F. E. HYTTEN

A possible influence of the type of carbohydrate included in the diet of animals upon cholesterol absorption and atherogenesis is an interesting observation. Further investigation of this action in man is awaited.

The Increased Severity of Atherosclerosis in Rabbits on a Lactose-Containing Diet. W. W. Wells and S. C. Anderson. *J. Nutrition*, 68: 541, 1959.

It had been reported that the absorption of cholesterol is increased in the rat by the addition of 40 per cent lactose to the diet. In this study, with rabbits, the diets consisted of 45 per cent soybean meal with additions of cottonseed oil, cod liver oil, vitamins and such, together with 0.35 per cent cholesterol. Lactose or sucrose was added to the test diets at 29.35 per cent.

The groups fed lactose consumed somewhat less food and finished the experiment somewhat lighter. Serum cholesterol values in the groups fed lactose averaged 100 to 180 mg. per 100 ml. higher than the control animals fed sucrose; differences began to be seen as early as two weeks. Total liver cholesterol concentrations of the groups fed lactose were more than double that of the animals fed sucrose. The "atherosclerosis score," as determined from measurements of plaques in the aortas, was higher also in the former. A good correlation was noted between serum cholesterol levels at two weeks and the eventual atherosclerosis scores.

The authors have no explanation for their findings. They eliminate the possibility that the influence of the lactose in the diet is related to a lowering of pH value in the cecum, or to a change in intestinal tract motility. Whether the known influence of lactose in producing a greater flow of lymph is related to cholesterol absorption is unknown. FRANK E. RICE

Coronary thrombosis is an event which appears, in some instances, to be related to the thrombophilic action of fats. This alteration in coagulability induced by certain fats may represent an important factor in the occlusion of coronary vessels affected by arteriosclerotic plaque formation.

The Independent Production of Atherosclerosis and Thrombosis in the Rat. G. A. Gresham, and A. N. Howard. *Brit. J. Exper. Path.*, 41: 395, 1960.

The development of thrombosis, myocardial and vascular lesions, and of obesity was studied in young male and female piebald rats. When they had reached a weight of 100 gm., the animals were fed diets up to 113 days varying in amounts of saturated and unsaturated fatty acids and their carbon number. The diet producing myocardial infarcts contained essentially 5 per cent cholesterol, 2 per cent cholic acid and 3 per cent thiouracil. Primarily the effects of butter fat and of arachis oil were tested, the latter rich in linoleic acid of low carbon number, the former rich in saturated fatty acids. Only the diet containing 40 per cent butter fat produced myocardial and renal infarcts due to thrombotic occlusion of the vessels. Substitution of 40 per cent butter fat by arachis oil failed to produce thrombi or infarcts; however, atherosclerosis was noted in the thoracic aorta and in the coronaries of rats thus fed. If the dose of arachis oil was reduced to 10 per cent, the atherosclerotic lesions were attenuated.

Rats fed 40 per cent butter fat or arachis oil only became obese but no vascular or myocardial lesions developed. All diets containing cholesterol and cholic acid produced arterial calcification with the presence of lipid-filled macrophages in the viscera. It is thus concluded that thrombosis, atherosclerosis and obesity are independent phenomena. M. SILBERBERG

Polyenic Acids in the Serum of Patients with Arteriosclerosis. G. Krickau and W. H. Hauss. *Ärztl. Forsch.*, 13: 187, 1959.

The question is examined as to how far alterations of the fatty acids of the serum, especially of highly unsaturated fatty acids, occur in persons with arteriosclerosis and metabolic diseases such as hyperlipemia, in comparison with healthy persons. The saturated fatty acids were separated from the unsaturated fatty acids by means of deep-freeze crystallization. The latter were measured up to the hexenes in the ultraviolet spectrum. In cases of hyperlipemia due to metabolic diseases, the saturated fatty acids in serum are clearly increased compared with the unsaturated ones. This was not the case in patients with arteriosclerosis. Among the polyenic acids the trienes were especially diminished in serum from arteriosclerotic subjects.

AUTHORS

Coronary Artery Disease and Obesity. K. Sanders. *Lancet*, 2: 432, 1959.

A group of forty-eight white male hospital patients with confirmed, uncomplicated coronary disease of recent onset were matched with a control group selected from a general practice. The control subjects were healthy men matched with the hospital group for age and for three factors which may have influenced dietary habits: nationality, place of living and religion. Body fat was estimated from a number of skinfold measurements in both groups.

The patients with coronary artery disease were no heavier than their control subjects but their skinfold thickness and therefore presumably their subcutaneous fat, was greater at all sites measured. These differences are said to be statistically significant but there is considerable overlap and such measurements would be of no diagnostic value in an individual subject.

F. E. HYTTEN

Inhibition of Progress of Preestablished Atherosclerosis by Diethylstilbestrol in the Rabbit. P. Constantinides and N. Gutmann-Auersperg. *Arch. Path.*, 70: 35, 1960.

Young male and female white New Zealand rabbits were fed a diet containing 1 per cent cholesterol, 5 per cent cottonseed oil, 90 mg./rabbit/day for nine weeks. At that time the degree of hypercholesteremia (1,315 to 1,372 mg. per 100 ml.) and plasma turbidity was determined. One batch of animals was sacrificed and the atherosclerotic involvement of aortas and of coronary arteries was graded. The remaining animals were observed for another eight weeks and treated as follows: (1) Feeding of 90 gm./rabbit/day of a stock diet, (2) feeding of 90 gm./rabbit/day of the stock diet and simultaneous subcutaneous injections of 2 mg./kg./body weight diethylstilbestrol in aqueous solution 3 times weekly, (3) feeding of the stock diet in amounts restricted to 40 gm./rabbit/day. Animals of group 1 showed no plasma turbidity and cholesterol values of 315 mg. per 100 ml., those of groups 2 and 3 showed slight plasma turbidity and cholesterol values of 465 and 466 mg. per 100 ml., respectively. In animals treated with the hormone the progress of atherosclerotic lesions was arrested in the aorta; but not in the coronary arteries. Underfeeding promoted the further development of atherosclerosis. M. SILBERBERG

Failure of Parenterally Administered Pyridoxine to Influence Serum Cholesterol Levels and Development of Atherosclerosis in Cholesterol-Fed Rabbits. F. W. Martens and D. W. Hoskins. *Circulation Res.*, 6: 159, 1958.

Twelve rabbits on a cholesterol-free diet and twelve on a 1 per cent cholesterol diet were given 25 mg. pyridoxine hydrochloride intramuscularly on alternate days for nine weeks. Twelve rabbits on a 1 per cent cholesterol diet and receiving phosphate buffer solution intramuscularly served as controls.

Serum cholesterol levels remained unaltered after three, six and nine weeks in all groups. Gross and microscopic examination of representative tissues from all animals after the ninth week showed no evidence that pyridoxine had any effect on the degree of atherosclerosis produced by cholesterol feeding.

W. H. ABELMANN